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A Journal of Pulmonary Diseases

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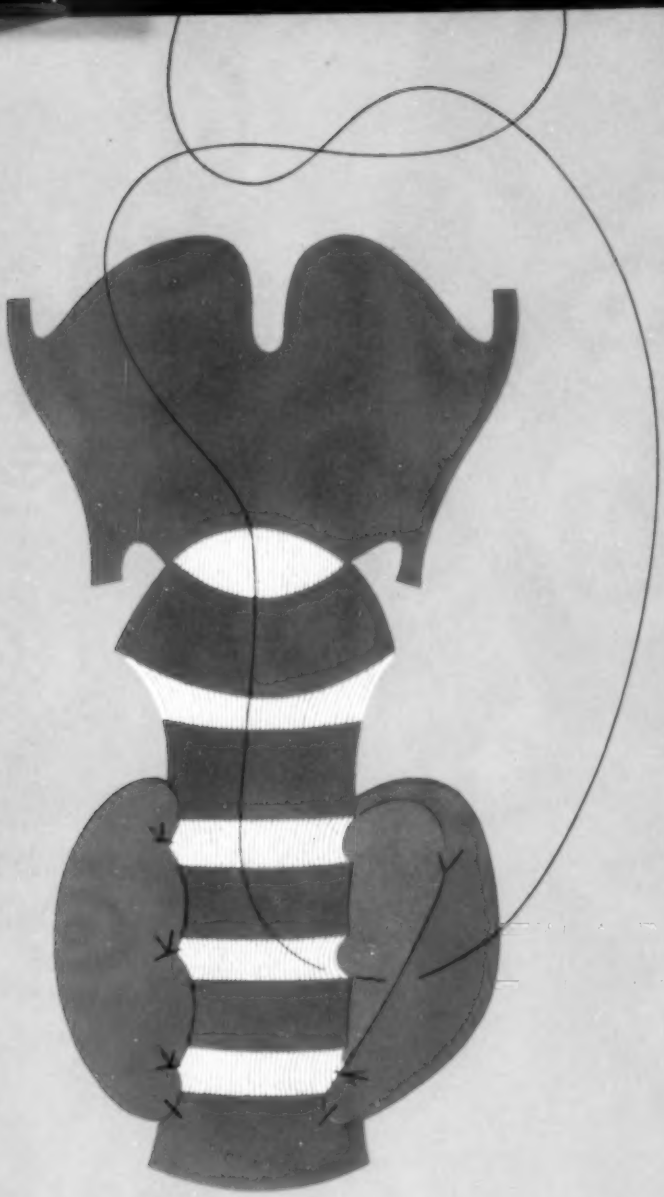

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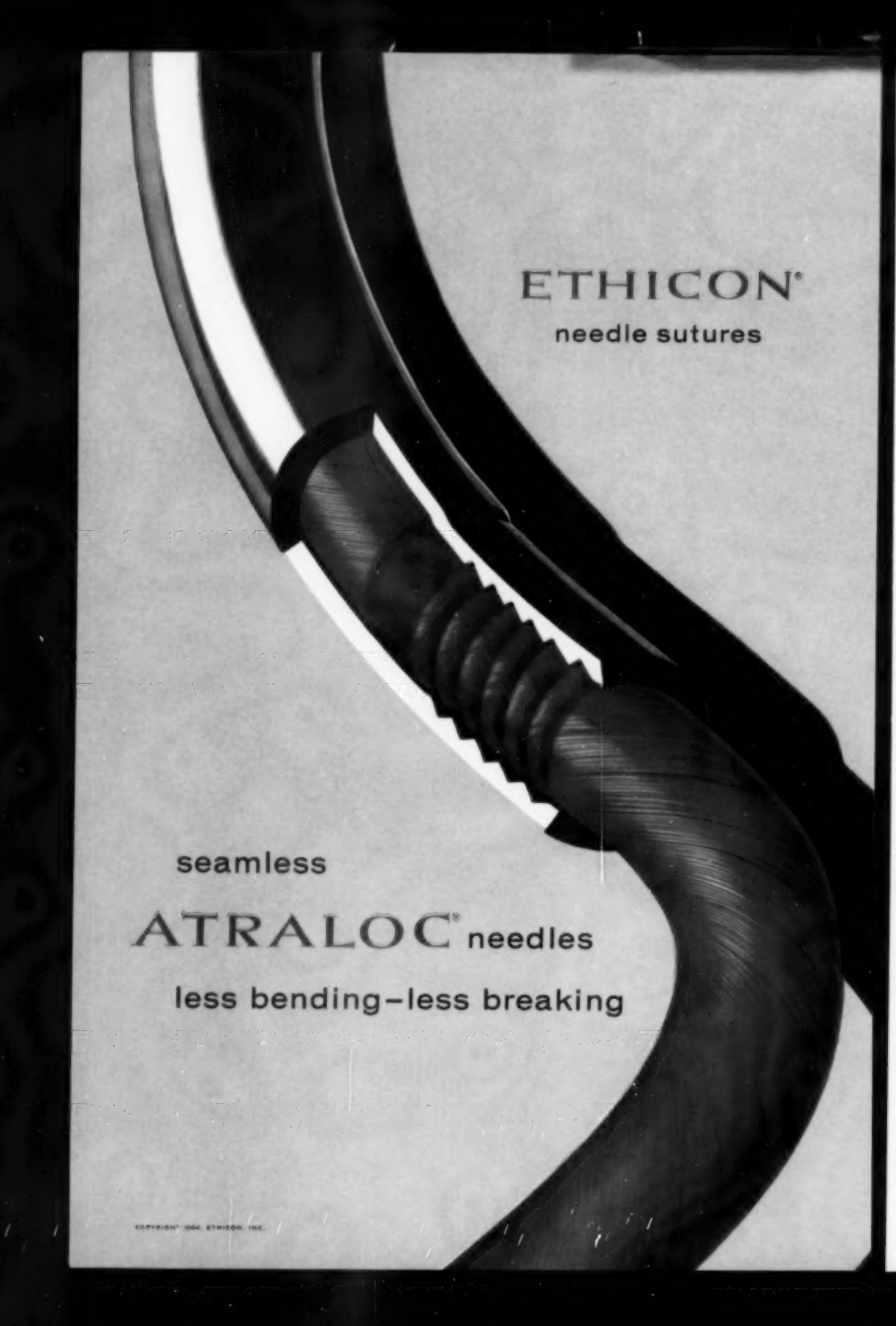
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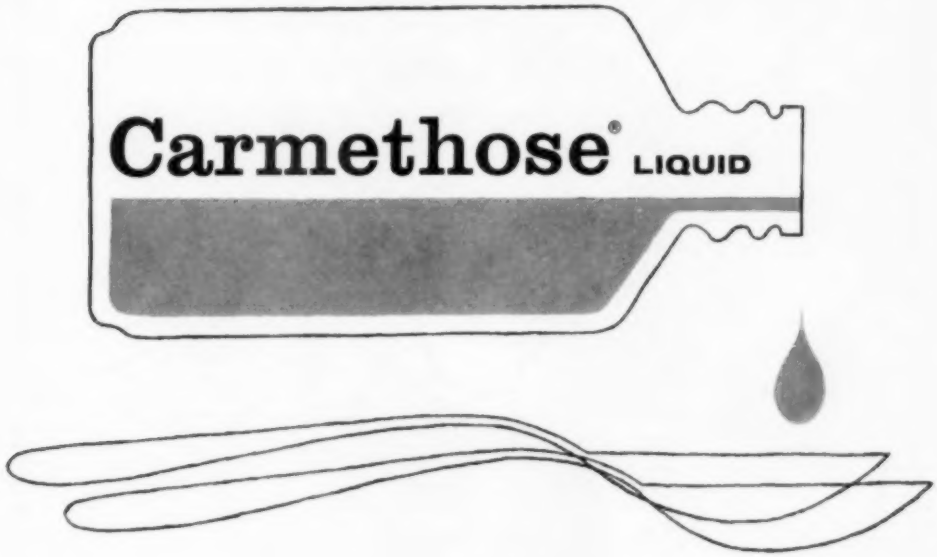

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1. Getz, H. R.; Westfall, I. S., and Henderson, H. J.: Nutrition in Tuberculosis as Evaluated by Blood Analysis, Am. Rev. Tuberc. *50*:96 (Aug.) 1944.
2. Getz, H. R.: Problems in Feeding the Tuberculous Patient, J. Am. Dietet. A. *30*:17 (Jan.) 1954.
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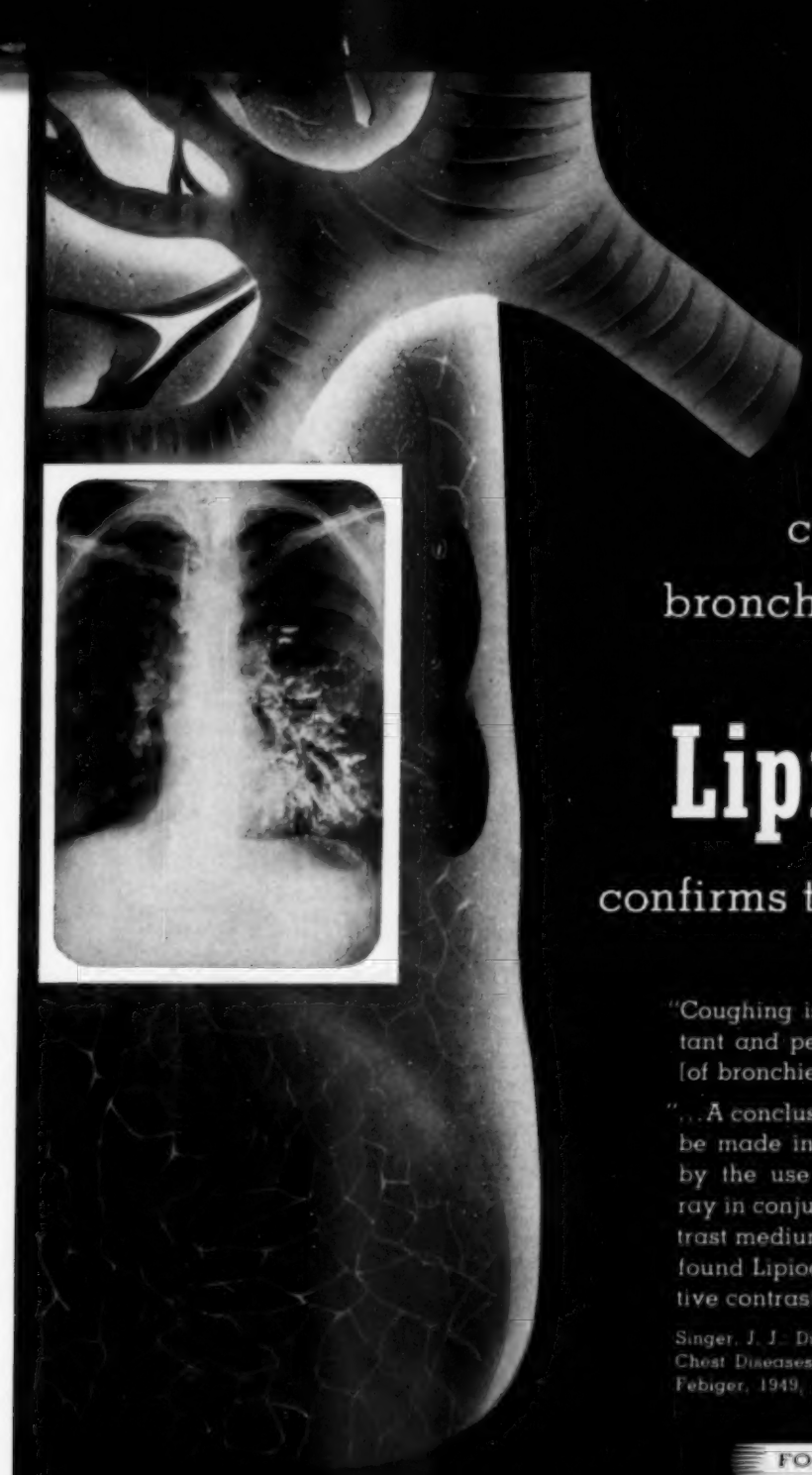

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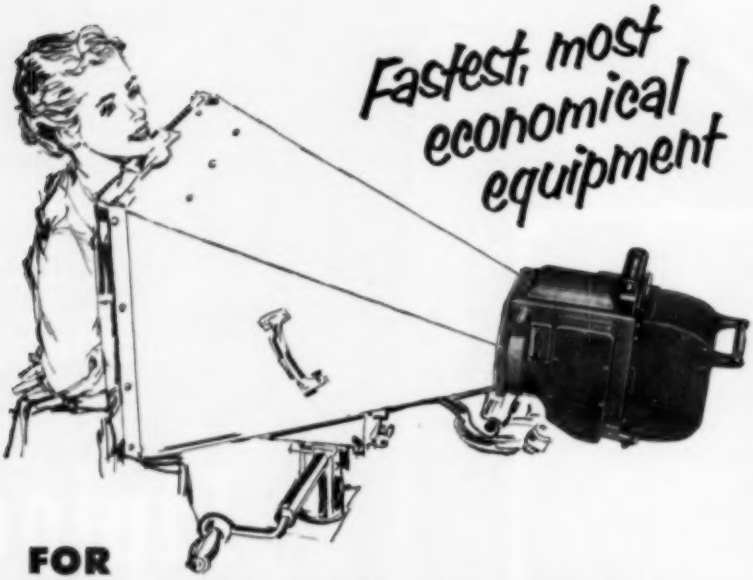

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ERRATA

In Volume 70, No. 2, 1954, "An Estimation of the Effective Alveolar Respiratory Surface and Other Pulmonary Properties in Normal Persons" by Russell H. Wilson *et al.*, equation II on page 297 should be changed to read:

$$R \quad 6.9 \times 10^4 \, (V/p) \, [(\log_{10} q_1 - \log_{10} q_2)/(t_2 - t_1)]$$

instead of:

$$R \quad 6.9 \times 10^4 \, (V/p) \, ((\log_{10} q_1 - \log_{10} q_2)/(t_2 - t_1))$$

to avoid the latter part of the equation being interpreted as an exponent of (V/p).

In Volume 70, No. 2, 1954, "Limitations of the Guinea Pig Test (Correspondence)" by Ernest H. Runyon, the second sentence on page 375 should be changed to read "There is no evidence, however, that *such* bacilli are necessarily avirulent for man" instead of "There is no evidence, however, that *tubercle* bacilli are necessarily avirulent for man."

THE AMERICAN REVIEW
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VOLUME 70 *October 1954* NUMBER 4

THE APICAL LOCALIZATION OF REINFECTION PULMONARY TUBERCULOSIS[1]

I. The Stream Flow Theory

DAVID T. SMITH, ROBERT S. ABERNATHY, GEORGE B. SMITH, JR., AND STUART BONDURANT

(Received for publication April 30, 1954)

INTRODUCTION

Clinicians have known for many generations that fibrocaseous pulmonary tuberculosis usually begins in the upper lobes. Various theories have been advanced to explain this phenomenon, but none have received general acceptance and there are many conflicts among them (1).

THEORIES FOR APICAL LOCALIZATION

The following statements are found in the literature:

1. The apex is less mobile than other parts of the lung; therefore, bacilli lodging there will remain.
2. The apex is more mobile than other portions of the lung and bacilli are drawn into it during respiration.
3. The apex is not as well aerated and, therefore, has less resistance to tuberculous infections.
4. The apex is better aerated and the tubercle bacilli are stimulated by the increased supply of oxygen.
5. The circulation of the apex is poor and the tissues accordingly less resistant.
6. The circulation is best at the apex and this results in hematogenous infections with a more favorable environment for development.
7. During cough, air from the lower lobes is expelled into the upper lobes, causing an accumulation of bacilli in the apices (2).
8. Inflammation and enlargement of the tracheobronchial and bronchopulmonary lymph nodes result in adhesions which anchor the hilum and thus limit the downward expansion of the upper lobes and, hence, favor the development of reinfection tuberculosis in the apices (2).

[1] From the Department of Bacteriology, School of Medicine, Duke University, Durham, North Carolina.

9. The apical bronchi form a right angle which impedes the movement of the inspired and expired air and favors the deposition of tubercle bacilli (3).

10. Because of the poor blood supply to the apices, the bronchial secretions are thicker and more viscid and bacilli lodging there are not as readily removed by coughing as from other portions of the lungs (3).

11. The relative lack of motility of the upper lobes, resulting in retention of exudate in the bronchi and bronchioles, is responsible for the prevalence of tuberculosis in this region and for its progression and chronicity (4).

12. The apices are infected by lymph drainage from the tonsils and cervical tissues.

13. A portion of the parietal pleura lying contiguous to the apices is drained by a network of lymphatics which empty into the deep cervical chain, and this causes a stasis of lymph flow and allows tubercle bacilli to lodge and develop (3).

Medlar and Sasano (5), in 1936, presented some experimental work which showed

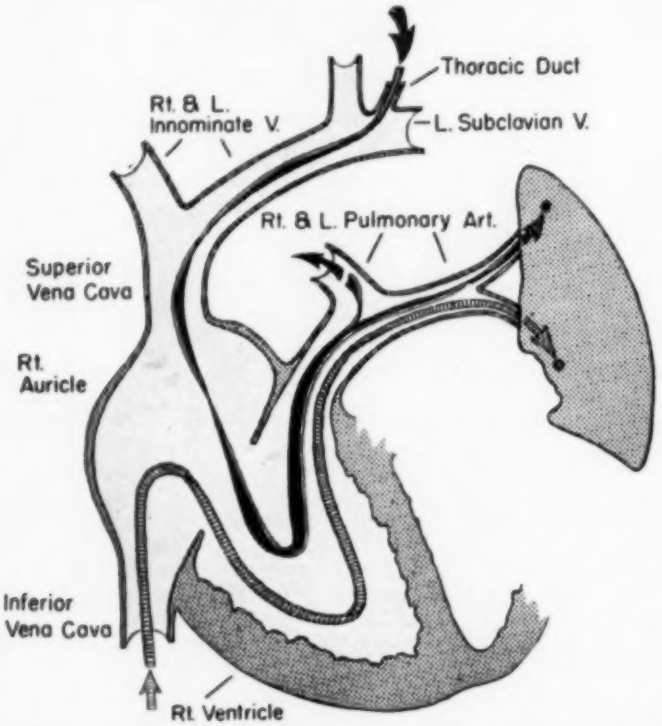

FIG. 1. A diagrammatic representation of the great venous vessels, the right auricle, right ventricle, and the pulmonary artery. The black ribbon-like band illustrates a type of "stream flow" which might carry caseous material, collected by the thoracic duct, through the right auricle and ventricle and on into the upper lobes of the lung. The cross-hatched ribbon illustrates a possible pathway for emboli from the pelvic and femoral veins to the lower lobes. There is some indirect but no direct evidence that this theoretical "layering" of the blood actually occurs.

conclusively that the number and extent of pulmonary lesions in the upper lobes of rabbits could be increased if the animals were forced to assume the upright position for eleven consecutive hours each day. This theory, which is No. 14, is sometimes called "Medlar's theory of position" or the "upright theory" and is the only one in the series backed by experimental evidence. A fifteenth theory was recently proposed by Dock (6, 7), which is in essence a combination of old theories Nos. 4 and 5. It assumes that the excess oxygen supply to the apices favors the growth of the tubercle bacilli while the sluggish blood flow to the upper lobes decreases the resistance of the tissues. Dock's figures, derived by calculations from actual measurements made during right heart catheterization studies, are very impressive. In the upright position, the pulmonic arterial pressure in the upper 5 cm. of the lungs can rarely be more than 10 mm. of mercury, and in tall thin persons must often be zero or even negative. Dock's calculations

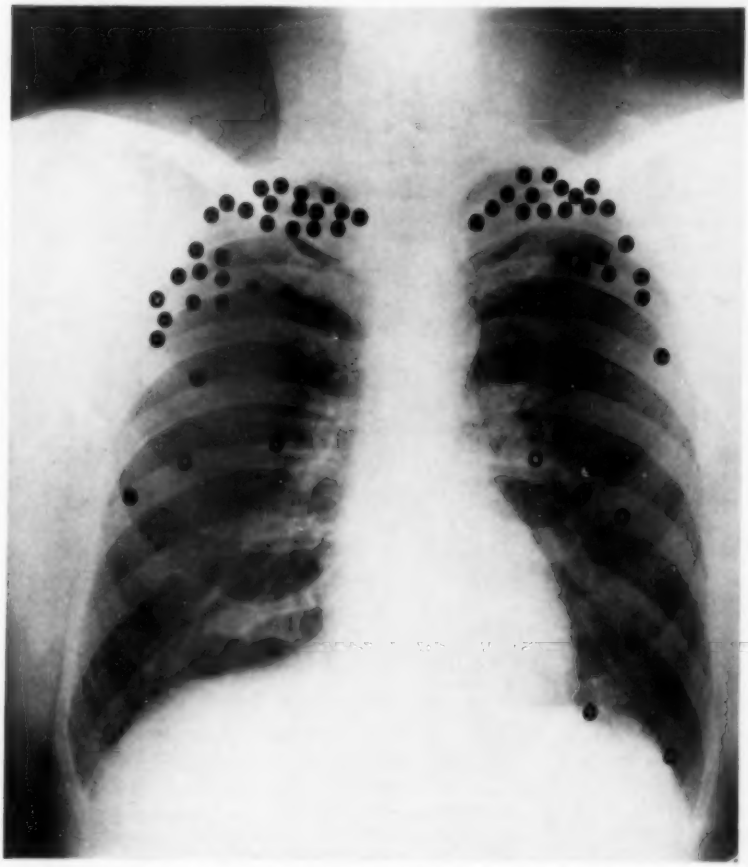

FIG. 2. Photograph of a roentgenogram of the chest on which has been charted the location of 55 lesions of the reinfection type of tuberculosis (after Medlar, Am. Rev. Tubere., 1948, *58*, 583).

of the hemodynamics of the pulmonary circulation support Medlar's and Sasano's conclusion that man's upright position favors the development of tuberculosis in the upper lobes. A sixteenth theory was proposed by Schwartz in 1953 (*7a*) which assumes that reinfection tuberculosis results from the rupture of an infected lymph node into a neighboring bronchus.

A NEW THEORY FOR APICAL LOCALIZATION

The present writers are introducing a seventeenth theory to explain the frequency of fibrocaseous tuberculosis in the upper lobes. This theory is based on

FIG. 3. Photograph of a roentgenogram of the chest on which has been charted the location, by lobes, of 100 consecutive infarcts. The original authors gave the location by lobes and not the location within the lobes. These have been indicated by the present authors in an arbitrary manner (after Hampton and Castleman, Am. J. Roentgenol., 1940, *43*, 305).

TABLE 1

LOCATION OF REINFECTION TUBERCULOSIS AND PULMONARY INFARCTS

Source of Data	Method of Study	Type of Lesion	Number of Lesions	Upper Lobe Lesions		Lower Lobe Lesions	
				Number	Per Cent	Number	Per Cent
Medlar	Necropsy	Pulmonary tuberculosis	68	60	88.2	8	11.8
Hampton and Castleman	Necropsy	Pulmonary infarcts	100	26	26.0	74	74.0

$P < 0.0001$.

a simple hydrodynamic principle that fluids of the same viscosity may flow side by side through tubes for a considerable distance with a minimum of admixture. It is assumed that the blood from the superior vena cava and that from the inferior vena cava do not have sufficient time to mix in the right auricle and right ventricle but pass through those chambers in layers in such a manner that most of the blood from the superior vena cava goes out in the superior portion of the pulmonary artery and, hence, by its superior branches to the upper lobes (figure 1). Conversely, blood from the inferior vena cava flows through the lower portion of the pulmonary artery and, hence, by inferior branches to the lower lobes. It is known that caseous material containing tubercle bacilli may be collected from various parts of the body, transported through the thoracic duct or right lymphatic duct, and emptied into the superior vena cava (8). It is assumed that these caseous particles are carried along in the stream flow through the heart and are deposited in the upper lobes as microscopic or macroscopic emboli. According to this "stream flow" theory, most reinfection or reactivation tuberculosis (9) should be in the upper lobes (figure 2), and most pulmonary infarcts, since they usually arise in the pelvic or leg veins and enter the heart through the inferior vena cava, should be in the lower lobes (figure 3, table 1).

This theory concerning the layering of the blood in its passage through the right auricle, right ventricle, and pulmonary artery was evolved by the senior author in 1929. Subsequent studies by other investigators have shown conclusively that there is a layering of the blood in the portal vein, part of it going to the right lobe and part to the left lobe of the liver (10).

DISCUSSION AND EVALUATION OF THEORIES

It is obvious from even a cursory review of these seventeen theories that in some it is assumed that the bacilli reach the lungs by inhalation (theories 1, 2 7, 9, 10, 11) and in others by way of the blood stream (theories 3, 4, 5, 6, 14, 15). Two theories require the occurrence of a lymphatic invasion from the cervical region (theories 12, 13); one, a rupture of a pulmonary lymph node into a bronchus (theory 16); and another, a blood stream invasion which is secondary to lymph drainage via the thoracic duct (theory 17).

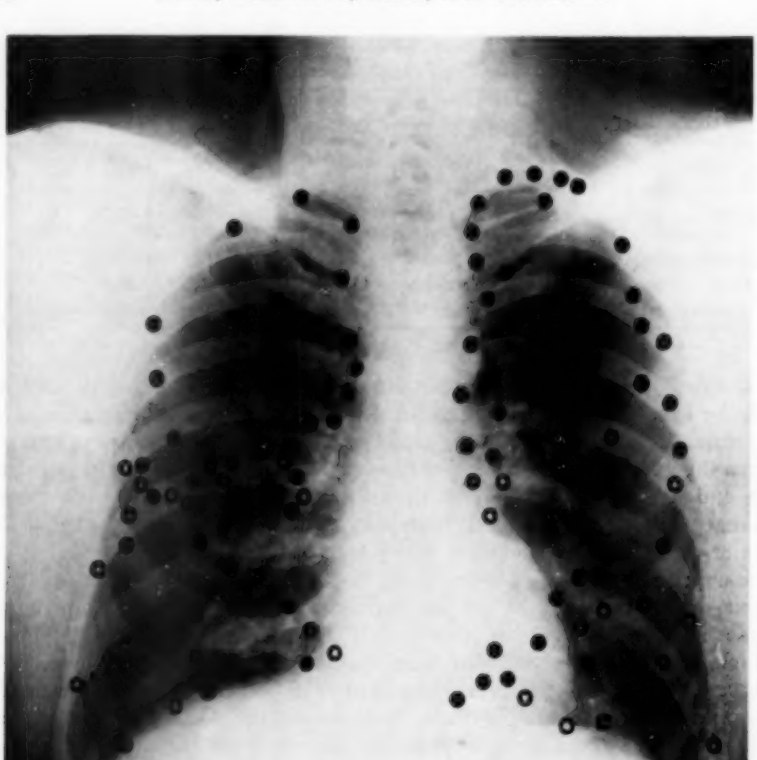

FIG. 4. Photograph of a roentgenogram of the chest on which has been charted the location of 105 lesions resulting from primary pulmonary infection with tubercle bacilli (after Medlar, Am. Rev. Tubere., 1948, *58*, 583).

The seventeen theories should be examined against the background of a few well-established facts concerning inhalation and hematogenous infections. There is general agreement that primary pulmonary tuberculosis usually results from the inhalation of tubercle bacilli. These lesions are not predominantly in the upper lobes but are found uniformly scattered throughout the lungs (figure 4). Furthermore, there is no evidence that those which happen to occur in the upper lobes heal any less readily than those in the lower lobes.

Gardner (11) has produced both progressive and retrogressive primary inhalation tuberculosis in guinea pigs. The mechanical distribution of the lesions was rather uniform and the healing, when it occurred, was of the same order regardless of the location of the lesion. The clinical, pathologic, and experimen-

tal evidence is all against the theories which assume that fibrocaseous tuberculosis in the apices results from the selective inhalation of tubercle bacilli. It should be noted that one of Jaffe's theories (No. 9) assumes a primary inflammation or fixation of the hilum before the mechanical conditions are satisfactory for localization in the apices by inhalation. It may be significant that the "upright position" of Medlar and Sasano and the demonstrably low, but assumed inadequate, pulmonary blood pressure in the apices (Dock) seem to have no particular deleterious effect on apical lesions of the primary inhalation type.

Infection of the cervical lymph nodes does not occur with sufficient frequency to explain the predominance of apical lesions, even if it could be shown that bacilli do pass directly from the cervical lymphatics to the lymphatics of the upper lobes. A direct rupture into a bronchus by a pulmonary lymph node can cause reinfection tuberculosis as has been demonstrated by Schwartz (7*a*), but this cannot explain upper lobe lesions when the primary infection is in another lobe, the neck, or abdomen.

The classical example of hematogenous tuberculosis is the ordinary type of miliary tuberculosis in which isolated bacilli or bacilli in small clumps are fed directly into the blood stream from a tuberculous abscess or caseous lymph node; or indirectly through the thoracic duct. Here the lesions are more or less uniform in size and distribution, not only in the lungs but also in the spleen, liver, bone marrow, and other organs. Spontaneous miliary tuberculosis is necessarily an endogenous reinfection or endogenous exacerbation. Spain (12) has reported that the miliary tubercles at the apices and in the upper part of the lower lobes may grow a little larger than those in other portions of the lung, but there is never complete resolution and disappearance of the basal lesions. Acute experimental miliary tuberculosis can be induced in animals at will by injecting a suspension of tubercle bacilli into the blood stream. In this instance also, the "miliary" lesions are rather uniform in size and distribution.

Somewhat more complicated is a second type of hematogenous tuberculosis, which cannot be called "miliary" because the lesions are of different sizes and apparently of different ages, even when occurring side by side in the same organ. These lesions may occur as a part of the picture of a progressive, primary infection. Some of the bacilli spread directly through the blood stream, while others invade the lymphatic system only to reappear in the blood as the caseous lymph nodes discharge their contents into the thoracic duct. Pinner (13) has observed that these nonmiliary hematogenous lesions may be larger and more numerous in the upper lobes. This type of slowly spreading tuberculosis can be reproduced by the subcutaneous injection of tubercle bacilli into the guinea pig or rabbit. If the dose is not excessive, the same type of disease can be produced by intravenous injection. At necropsy, the tuberculous lesions are found to be both larger and more numerous in the lower lobes than in the upper lobes (14, 15).

This same selective localization of the tuberculous lesions has been found in spontaneous tuberculosis in cattle and other animals (14). Having observed that the so-called "lower lobes" in the rabbit and cow are actually superior in position to the so-called "upper lobes," Medlar and Sasano evolved their "up-

right theory," in which they assumed that tuberculosis develops most readily in the superior portion of the lungs, although the actual original seeding of bacilli through the blood stream may have been uniform throughout the lungs. To test their theory, a group of rabbits were inoculated intravenously through the ear vein with a suspension of tubercle bacilli. Nine rabbits were fitted with a corset-like arrangement about the thorax and abdomen so that the animals were forced to sit upright on their haunches for eleven consecutive hours each day for more than three months. In these "upright" rabbits, the lesions in the upper lobes were larger and more numerous. Medlar and Sasano assume that all of the lobes were seeded uniformly with bacilli, and the location of the larger lesions was determined entirely by the position of the animal. While the original seeding may have been uniform, evidence will be presented in the next article in this series to show that the subsequent spreads back to the lungs may have been influenced by the "stream flow" in such a manner as to produce the larger lesions in the upper lobes of the upright rabbits and in the lower lobes in the horizontal animals.

Dock's theory also assumes a uniform spread of infection with specific development in the apices when the pulmonary pressure is low. It should be emphasized that a low pulmonary pressure of the apices is not necessarily an inadequate pressure, and a high pressure in the lower lobes does not necessarily produce an environment unfavorable for the development of tuberculosis. Several of Dock's assumptions have already been challenged. Patients with mitral stenosis and high apical pressures do develop apical tuberculosis (12), and patients with tuberculosis confined to the lower lobes show neither a decreased nor increased resistance to the disease (16).

SUMMARY

Sixteen theories have been found in the literature as explanations for the predominantly apical localization of reinfection pulmonary tuberculosis. These have been reviewed, discussed, and evaluated from a clinical point of view. Those theories which assume that the bacilli reach the upper lobes by inhalation are obviously incorrect since they are not supported by either experimental data or clinical observations. Lymphatic infections in the cervical region with a spread of the organisms from the neck to the upper lobes must be a rare accident, if it occurs at all. There remains the blood stream as the medium by which tubercle bacilli reach the upper lobes in reinfection tuberculosis. This mechanism has been assumed in six of the sixteen theories, but no adequate explanation has been offered for the failure of lesions to appear simultaneously in the lower lobes.

In the theory proposed by Medlar and Sasano, it is assumed that both upper and lower lobes are seeded uniformly but the bacilli disappear from the lower lobes without producing detectable lesions, while progressive lesions develop in the upper lobes as a result of an "inadequate circulation" dependent upon the "upright" position of man. The experiments performed by Medlar and Sasano seem to prove their theory since rabbits which were forced to sit upright for eleven hours per day for more than three months had more lesions and larger

lesions in the upper lobes. The experiments of Medlar and Sasano have been repeated and confirmed by Dr. Stuart Bondurant in this laboratory. Although the observations of Medlar and Sasano have been confirmed, a different interpretation has been made of the results (17). In the next article in this series, experimental data are presented which seem to show that the upper lobes are infected directly and immediately as a result of the stream flow through the heart. The lesions do not depend upon an inadequate circulation but are dependent upon the vertical, upright, or "upside down" position of the animal since the position of the animal alters the stream flow through the heart.

SUMARIO

La Localización Apical de la Tuberculosis Pulmonar de Reinfección: I. La Teoría del Flujo de la Corriente

En la literatura, se han encontrado diez y seis teorías que tratan de explicar el predominio de la localización apical en la tuberculosis pulmonar de reinfección. Todas ellas han sido repasadas, discutidas y justipreciadas desde el punto de vista clínico. Las teorías que presuponen que los bacilos llegan a los lóbulos superiores por inhalación son manifiestamente erróneas, por no prestarles apoyo ni los datos experimentales ni las observaciones clínicas. Las infecciones linfáticas en la región cervical con difusión de los microbios desde el cuello a los lóbulos superiores debe constituir un accidente raro, si es que ocurre. Esto deja a la circulación sanguínea como medio por el cual los bacilos tuberculosos llegan a los lóbulos superiores en la tuberculosis de reinfección. Este mecanismo se da por sentado en seis de las diez y seis teorías, pero no se ha ofrecido explicación adecuada de la falta de aparición simultánea de lesiones en los lóbulos inferiores.

En la teoría propuesta por Medlar y Sasano, se presupone que quedan sembrados por igual los lóbulos superiores y los inferiores, pero que los bacilos desaparecen de los últimos sin producir lesiones descubribles mientras que se presentan lesiones evolutivas en los lóbulos superiores a consecuencia de una "circulación inadecuada" debida a la posición "erecta" del hombre. Los experimentos ejecutados por Medlar y Sasano parecen confirmar su teoría, dado que los conejos a los que se obligó a permanecer sentados verticalmente durante once horas al día por más de tres meses tuvieron más lesiones, y éstas mayores, en los lóbulos superiores. Los experimentos de Medlar y Sasano han sido repetidos y confirmados por el Dr. Stuart Bondurant en el laboratorio de los AA. Aunque las observaciones de Medlar han quedado corroboradas, no cabe aceptar sus conclusiones. En el próximo trabajo de esta serie, se presentarán datos experimentales que parecen demostrar que los lóbulos superiores se infectan directa e inmediatamente a consecuencia del flujo de la corriente de sangre a través del corazón. Las lesiones no se deben a una circulación inadecuada sino a la posición vertical, erecta o virada de arriba para abajo del animal, dado que la posición de éste altera el flujo de la corriente por el corazón.

RESUME

La localisation apicale de la tuberculose de réinfection: I. Théorie du courant sanguin

Sieze théories pour expliquer la prédominance de la localisation apicale de la tuberculose pulmonaire de réinfection ont été découvertes dans la littérature. Celles-ci sont passées en revue, discutées et évaluées du point de vue clinique. Celles des théories qui admettent que les bacilles atteignent les lobes supérieurs par inhalation sont évidemment fausses, car elles ne sont pas confirmées par les données expérimentales ou les observations cliniques. Les infections lymphatiques de la région cervicale, avec dissémination de bacilles, de la région cervicale vers les lobes supérieurs, doivent être un accident rare, s'il existe. Il reste à considérer le courant sanguin comme étant le milieu par lequel les bacilles tuberculeux atteignent les lobes supérieurs dans la tuberculose de réinfection. Six des sieze théories émises admettent l'hypothèse de ce mécanisme, mais aucune explication adéquate

de l'absence d'apparition de lésions simultanées dans les lobes inférieurs n'a été formulée.

Dans la théorie proposée par Medlar et Sasano, il est admis que les lobes supérieurs et inférieurs sont tous deux inoculés uniformément, mais que les bacilles disparaissent des lobes inférieurs sans y produire de lésions décelables, tandis que des lésions progressives se développent dans les lobes supérieurs en raison d'une "circulation inadéquate" résultant de la position "debout" de l'homme. Les expériences effectuées par Medlar et Sasano ont été répétées et confirmées par le Dr. Stuart Bondurant dans notre laboratoire. Bien que les observations de Medlar aient été confirmées, les auteurs déclarent qu'ils ne peuvent en accepter les conclusions. Ils présenteront, dans un article suivant de cette série, leurs données expérimentales, qui paraissent indiquer que les lobes supérieurs sont infectés directement et immédiatement par le courant sanguin traversant le coeur. Ces lésions ne dépendent pas d'une circulation inadéquate, mais elles sont conditionnées par la position verticale, debout ou tête en bas de l'animal, car la position de l'animal modifie le courant du sang traversant le coeur.

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THE APICAL LOCALIZATION OF REINFECTION PULMONARY TUBERCULOSIS[1]

II. Selective Localization of Experimental Emboli

ROBERT S. ABERNATHY, GEORGE B. SMITH, JR., AND DAVID T. SMITH

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INTRODUCTION

The various theories which purport to explain the apical localization of reinfection pulmonary tuberculosis have been reviewed and discussed in the first paper of this series (1).

The theory proposed by the present writers assumes that pus or small particles of caseous material are discharged from infected lymph nodes and carried by the lymph vessels through the thoracic duct to the left subclavian vein to join the blood entering the heart from the superior vena cava. It is assumed that the blood from the upper part of the body will pass through the right auricle, right ventricle, and into the pulmonary artery with little, if any, mixing with the blood entering the heart from the inferior vena cava.

This "stream flow" theory can be studied in animals, at least to the extent of proving or disproving that blood entering the heart from the superior vena cava does not mix thoroughly with that from the inferior vena cava while in the right auricle or right ventricle, and in effect is distributed to a specific portion of the lung through the branches of the pulmonary artery. If this is true, then particulate matter capable of blocking pulmonary capillaries or arterioles, when injected into the ear vein of a rabbit, should produce infarcts in one portion of the lungs, while the same type of material introduced into the blood stream through the femoral vein should induce infarcts in a different portion of the lung. As the rabbit, under normal experimental conditions, develops the larger and more numerous tuberculous lesions in the lower lobes, it would be anticipated that the blood entering the rabbit's heart from the superior vena cava would be directed largely to the lower lobes and, therefore, particulate material introduced into the ear vein of a rabbit should produce infarcts in the lower lobes. Conversely, particulate material introduced into the femoral vein should induce infarcts in the upper lobes.

MATERIALS AND METHODS

Experimental infarction with Lycopodium spores: After considering a number of inert, nontoxic, particulate materials, the spores of the club moss *Lycopodium* were chosen. They are quite uniform in size, range from 40 to 50 microns in diameter, and have been used previously for the production of pulmonary embolism and infarction (2).

[1] From the Department of Bacteriology, School of Medicine, Duke University, Durham, North Carolina.

The spores were stained so that they could be recognized readily in the lungs. For staining, the spores were placed in 95 per cent ethyl alcohol containing crystalline nile blue sulfate dye in an open dish and allowed to stand until all the alcohol had evaporated. The excess dye was removed by washing with distilled water, and the stained spores were again washed with a small amount of ethyl alcohol, for it was found that this gave a more uniform suspension. For injection purposes, the spores were suspended in pooled, normal human sera, a more physiologic medium than water or saline. A suspension, 5 or 10 per cent by weight, could easily be obtained by gentle rotation; the suspension proved to be fairly uniform, and the spores showed only a slight tendency to settle out. However, as it was noticed that the spores tended to settle gradually over a long period, no suspensions more than one week of age were used for injection purposes. The spores in suspension tended to form small clumps, and it was necessary to use a No. 20 needle for injection. The clumping effect proved desirable, however, as the clumps gave larger emboli.

After some preliminary experiments with several animals, it was found that the optimal dose of the spore suspension was 0.05 ml. Larger doses gave scattering of emboli in both upper and lower lobes without any localization; while, with smaller doses, lesions were produced infrequently. Even with the 0.05 ml. dose, an occasional animal failed to show lesions. Accordingly, each series of animals includes more than the number shown, since only those animals in which lesions were produced are reported in the study. Rabbits were used because of comparatively large veins and their easy availability.

In the preliminary experiments, it was noted that the emboli caused scattered, usually small but sometimes relatively large, hyperemic areas at the pleural surfaces of the lungs. These varied from a few millimeters to a third of a lobe in size. They were almost invariably located on the dorsal pleural surface but occasionally would be found on the ventral surface. Typically, these hyperemic areas were found at the periphery of the lung and were often wedge-shaped although there was considerable variation in the shape. They were often unilateral but sometimes confined to a single lobe. Microscopic study of sections taken from these grossly hyperemic areas showed hemorrhage in the pulmonary parenchyma, but little, if any, necrosis such as is usually seen in a pulmonary infarction. As the animals were routinely sacrificed after one day, however, there would hardly have been sufficient time for necrosis to occur. These areas undoubtedly represented vascular occlusion by the spores, which were readily visible in the smaller arteries, arterioles, and capillaries, some singly, some in clumps, and others lined up in a vessel. The lobe in which the embolus had lodged could be determined from the location of the hyperemic area. In some instances the infarcted areas were quite faint and difficult to locate when the lungs were first removed, but after standing at room temperature for ten to twenty minutes they became definitely red and easy to outline. Immediately after the lungs were fixed by the intratracheal injection of 10 per cent formalin, the lesions were sometimes more distinct and sometimes less distinct.

In the first series of experiments, the spores were injected sometimes in the ear vein and sometimes in the femoral vein to see if the route of injection would localize lesions in the upper or lower lobes. In the first group, the rabbit was placed in an ordinary rabbit box, the ear was shaved, and 0.05 ml. of the spore suspension was injected through the marginal ear vein. The rabbit was retained in its normal horizontal position in this experiment. The animals were sacrificed the following day by rapid intravenous injections of an overdose of sodium

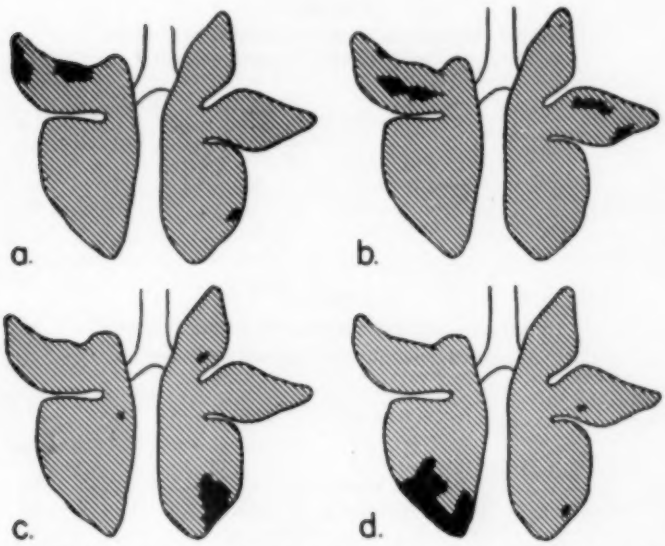

FIG. 1. *a*. Two infarcts in an upper lobe and one in the lower lobe of a rabbit's lung. Lesions induced by the injection of *Lycopodium* spores into the femoral vein of a rabbit. *b*. Infarcts in the upper lobes induced by the injection of tuberculous pus in the femoral vein of a rabbit. *c*. Large infarct in a lower lobe and a small one in an upper lobe. Lesions induced by the injection of *Lycopodium* spores into the ear vein of a rabbit. *d*. Large infarct in a lower lobe and a small one in a middle lobe induced by the injection of tuberculous pus into the ear vein of a rabbit.

pentothal. Chloroform and ether anesthesia were found unsatisfactory because they produced irritation which sometimes obscured the lesions produced by the spores. The lungs were dissected out and inspected for gross lesions. Each lesion was plotted on an outline chart of the lungs (figure 1).

RESULTS

In the first group of animals, the *Lycopodium* spores were injected in the ear vein. A total of 38 lesions was produced in 11 rabbits; of these, 31 (81.6 per cent) were located in the lower lobes, while only 7 (18.4 per cent) were in the upper lobes (table 1).

In the second group of animals, the femoral vein was used as the site of injection.

> The rabbit was anesthetized carefully with intravenous sodium pentothal, then placed flat on its back, with the hind legs spread out and tied down. The femoral region was shaved, and after incision of the overlying skin the femoral vein was isolated from the nerve and artery by blunt dissection. The spore suspension, 0.05 ml., was then injected with a No. 20 needle, after which ligatures were tied above and below the point of injection and the needle withdrawn. The skin incision was

TABLE 1

SELECTIVE LOCALIZATION OF PULMONARY EMBOLI
Infarcts Produced with *Lycopodium* Spores

Route of Injection	Position of Rabbits	Number of Infarcts	Upper Lobe Infarcts		Lower Lobe Infarcts	
			Number	Per Cent	Number	Per Cent
Ear vein	Normal (horizontal)	38	7	18.4	31	81.6
Femoral vein	Back (horizontal)	52	27	51.9	25	48.1

$P < 0.002$.

TABLE 2

SELECTIVE LOCALIZATION OF PULMONARY EMBOLI
Infarcts Produced with *Lycopodium* Spores

Route of Injection	Position of Rabbits	Number of Infarcts	Upper Lobe Infarcts		Lower Lobe Infarcts	
			Number	Per Cent	Number	Per Cent
Ear vein	Back (horizontal)	73	17	23.3	56	76.7
Femoral vein	Back (horizontal)	52	27	51.9	25	48.1

$P < 0.001$.

then closed and the animal returned to its cage until the following day, when it was sacrificed with an overdose of pentothal. The lungs were dissected out, and the gross lesions were recorded on a chart (figure 1).

A total of 52 lesions was produced in 8 rabbits; of these, 27 (51.9 per cent) were in the upper lobes, while 25 (48.1 per cent) were in the lower lobes (table 1). This is a marked difference from the results obtained with injection through the ear vein. In addition, the lesions in the femoral vein group were larger and more extensive in the upper lobes when compared with those in the lower lobes; on the other hand, in the ear vein group, the lesions in the lower lobes were more extensive. When the results shown in table 1 were subjected to statistical analysis, it was seen that the differences were significant, having a P value of <0.002.

In the two series of experiments reported above, the rabbits were in the horizontal position but the rabbits in one group were lying on their backs while those in the other group were standing in the normal position. To control this variation, a series of injections was given in the ear vein with the animals lying on their backs. The lesions were predominantly in the lower lobes when the *Lycopodium* seeds were injected in the ear vein (tables 2 and 3).

The Effect of Position of the Animal on the Location of Experimental Infarcts

Medlar and Sasano (3) have shown that rabbits develop more and larger tuberculous lesions in the upper lobes after intravenous inoculation of tubercle bacilli into the ear vein when they are kept in the upright position for eleven

TABLE 3

SELECTIVE LOCALIZATION OF PULMONARY EMBOLI

Infarcts Produced with *Lycopodium* Spores

Route of Injection	Position of Rabbits	Number of Infarcts	Upper Lobe Infarcts		Lower Lobe Infarcts	
			Number	Per Cent	Number	Per Cent
Ear vein.....	Normal and back (horizontal)	111	24	21.62	87	78.38
Femoral vein....	Back (horizontal)	52	27	51.9	25	48.1

$P < 0.001$.

TABLE 4

SELECTIVE LOCALIZATION OF PULMONARY EMBOLI

Infarcts Produced with *Lycopodium* Spores

Route of Injection	Position of Rabbits	Number of Infarcts	Upper Lobe Infarcts		Lower Lobe Infarcts	
			Number	Per Cent	Number	Per Cent
Ear vein.....	Normal (horizontal)	38	7	18.4	31	81.6
Ear vein.....	Back (horizontal)	73	17	23.3	56	76.7
Ear vein.....	Upright	44	18	40.8	26	59.2
Ear vein.....	Inverted	42	17	40.5	25	59.5

consecutive hours each day. These investigators attributed the result to a selective development of the lesions in the upper lobes and assumed that there would always be a uniform distribution of the infecting material to all lobes. The present writers questioned this assumption because the experiments reported above show that particulate material is not distributed evenly to the lungs but varies, depending specifically upon the route of inoculation, ear vein or femoral vein. In the next series of experiments, all inoculations of *Lycopodium* spores were made through the ear veins so that the only variable would be the position of the rabbit at the time of inoculation. Immediately after inoculation, the animals were returned to their cages and assumed the normal horizontal position.

The rabbits were packed in an ordinary rabbit box with excelsior so that they could not move or shift their position, and then the box was placed in different positions. Four different positions were used: (*1*) *upright*, in which the box stood on end with the head of the rabbit uppermost; (*2*) *inverted*, in which the box was again on end, but with the rabbit's head down and the caudal portion uppermost; (*3*) *back*, in which the box was placed so that the rabbit was on its back. In addition, those rabbits injected in the normal *horizontal* position were used in this series. The results are tabulated in table 4.

There was apparently little difference in the localization of emboli between the two horizontal positions back and normal. This was confirmed by statistical analysis of the results in these two groups; '*t*', in this case, was found to be 0.54. Thus, the small difference observed could have been due to chance. The same

TABLE 5

SELECTIVE LOCALIZATION OF PULMONARY EMBOLI

Infarcts Produced with *Lycopodium* Spores

Route of Injection	Position of Rabbits	Number of Infarcts	Upper Lobe Infarcts		Lower Lobe Infarcts	
			Number	Per Cent	Number	Per Cent
Ear vein.....	Normal and back (horizontal)	111	24	21.62	87	78.38
Ear vein.....	Upright and inverted (vertical)	86	35	40.70	51	59.30

$P < 0.003$.

was true for the two vertical positions. The results here were almost identical. There was, however, a sharp difference in the localization between the vertical positions and the horizontal positions. If the results for the two horizontal positions are added and compared with the sum of the results for the two vertical positions, this difference is confirmed by statistical analysis (table 5).

Pulmonary Infarcts Produced with Tuberculous Pus

Because of encouraging results in influencing the localization of pulmonary emboli with the use of *Lycopodium* spores, it was decided that a more physiologic embolus was advisable, particularly one which would be analogous to those derived from the reactivation of an old tuberculous focus in a lymph node.

Pus from a tuberculous cold abscess is the most readily available source of such material. For this purpose, rabbits were inoculated subcutaneously in the axillary region with 0.3 to 0.4 ml. of a cloudy suspension of R1v tubercle bacillus, which is nonpathogenic except for silicotic guinea pigs. After several weeks the rabbits developed abscesses, associated with a mild enlargement of the regional lymph nodes. The pus thus formed proved to be too thick and pasty for withdrawal through a needle and syringe and consequently had to be obtained by incision of the abscess. A smear of the creamy thick pus invariably revealed many acid-fast bacilli. As obtained from the abscess, the pus was too thick for injection through a needle and was diluted with sterile physiologic saline to a consistency which would allow injection. As this pus was ejected through the needle, it could be seen to form an embolus, the size of which was limited by the diameter of the 20-gauge needle; it usually averaged approximately 1 cm. in length when the customary dosage of 0.05 ml. was used.

The pulmonary lesions produced by the pus resembled grossly those produced by the spore suspension and were noted to have the same predominance of a dorsal subpleural location, frequently at the edges of the lobes. For the most part, the lesions as observed macroscopically were areas of hyperemia, but on one occasion there was evident gangrene of the base of a lower lobe. It was found that the lesions were best seen if the animals were allowed to live three or four days after the injection and were then sacrificed. Some of the rabbits used were sensitized to R1 tubercle bacilli by virtue of the subcutaneous inoculation for purposes of obtaining pus; these animals could have had a tuberculin-like reaction from the tuberculous

pus injected. Nevertheless, there was little difference in the gross appearance of lesions produced in these sensitized animals as compared with those in the normal, nonsensitized rabbit. Microscopic study of sections taken from typical lesions showed a varied picture. In several cases in which the gross lesion consisted of a diffuse hyperemia (in one case in the lower lobe and in another in the upper lobe), there was a diffuse nodular epithelioid reaction which closely resembled a fresh tu-

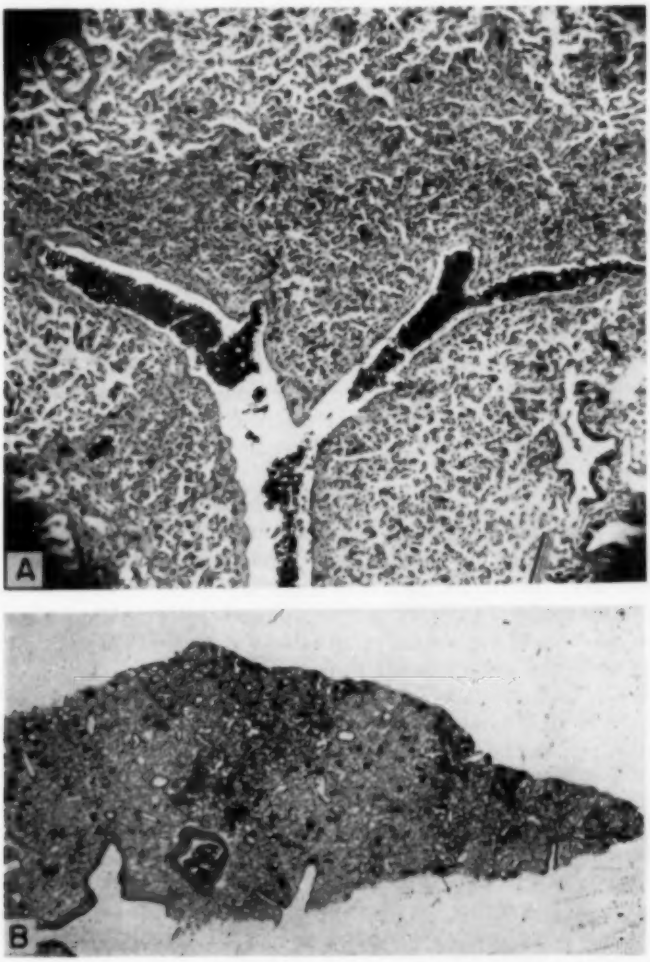

FIG. 2. *A*. Photomicrograph of a selected area showing a thrombus in a pulmonary artery surrounded by a mononuclear cellular infiltration induced by the intravenous injection of tuberculous pus. *B*. Low-power photomicrograph showing a thrombus in a pulmonary artery, a wedgelike infarcted area, and isolated areas of infiltration. Lesions were produced by the intravenous injection of tuberculous pus.

berculous process, although no acid-fast organisms were found in the sections (figure 2*A*). Another lung with much the same gross characteristics showed a moderate interstitial cellular infiltration, consisting predominantly of mononuclear cells (figure 2*B*). In two other instances, from femoral vein injection, there were firm, meatlike, shrunken linear lesions resembling atelectasis in the upper lobes; microscopic examination confirmed the presence of atelectasis without the presence of acid-fast organisms. Whether the lesions visible grossly were tuberculous in origin or were the result of vascular occlusion by the pus emboli is difficult to determine. The short period of time between the injection of the tubercle bacilli-containing pus and the sacrificing of the animal would seem to favor the latter explanation; in favor of the former is the epithelioid reaction seen on several sections. Roentgenograms of the inflated isolated lungs in more than 10 of the rabbits did not consistently confirm the presence of lesions grossly visible. In one of the animals injected while in the horizontal position, there was a gross lesion at the base of the right lower lobe which showed a mottled lesion on roentgenography; histologically, there were scattered nodules of epithelioid cells as described above. The atelectatic areas mentioned above could also be seen on the roentgenogram.

With the tuberculous pus as an embolus, several groups of rabbits were injected while being held in various specific positions. One group of animals were injected in the ear vein while being on their backs in a horizontal position. A total of 43 lesions was produced in 10 animals; 33 (76.75 per cent) of these were in the lower lobes, while 10 (23.25 per cent) were in the upper lobes (table 6). Another series of animals, in the same position, received the tuberculous pus in the femoral vein. The technique of injection was as described for the spore suspension.

A total of 45 lesions was produced in 14 rabbits; of these, 24 (53.4 per cent) were in the upper lobes, while 21 (46.6 per cent) were in the lower lobes. This is in marked contrast to the distribution in animals which received the injection in the ear vein. The results parallel closely those obtained with the spore suspension (table 2).

In the other positions, a total of 10 rabbits was used. The total number of lesions in the entire series injected through the ear vein was 64; 50 (78.15 per cent) were in the lower lobes and 14 (21.85 per cent) were in the upper lobes.

As shown in table 6, more lesions were produced in the upper lobes when the tuberculous pus was injected into the femoral vein while the rabbits were lying

TABLE 6

SELECTIVE LOCALIZATION OF PULMONARY EMBOLI

Infarcts Produced with Tuberculous Pus

Route of Injection	Position of Rabbits	Number of Infarcts	Upper Lobe Infarcts		Lower Lobe Infarcts	
			Number	Per Cent	Number	Per Cent
Ear vein	Back (horizontal)	43	10	23.25	33	76.75
Femoral vein	Back (horizontal)	45	24	53.4	21	46.6

$P < 0.003$.

TABLE 7

SELECTIVE LOCALIZATION OF PULMONARY EMBOLI

Infarcts Produced with Tuberculous Pus

Route of Injection	Position of Rabbits	Number of Infarcts	Upper Lobe Infarcts		Lower Lobe Infarcts	
			Number	Per Cent	Number	Per Cent
Femoral vein	Back (horizontal)	45	24	53.4	21	46.14
Femoral vein	Inverted (vertical)	82	26	31.7	56	68.3

$P < 0.02$.

on their backs in the horizontal position. In the next experiment, the pus was injected into the femoral vein and the animals were immediately tipped into the vertical position with the head down. This shift in the position of the rabbit increased in a significant manner the number of lesions in the lower lobes (table 7).

DISCUSSION

The results of the above experiments show that both the route of injection and the position of the animal have a definite effect on the localization of pulmonary emboli. When the rabbit is in either of the horizontal positions, the large majority of lesions produced by pulmonary embolism are in the lower, or caudal, lobes on the dorsal pleural surface. This corresponds to the same portion of the lung field which Medlar found to be so predominantly involved in pulmonary tuberculosis of both the rabbit and cow. On the other hand, when the animal is shifted to one of the vertical positions, there is a significant increase in the number of lesions in the upper lobes. This result is in line with Medlar's findings of a shift in predominance of tuberculous lesions to the anterior superior lung fields when rabbits were held in the upright, or vertical, position for long periods of time.

As posture does have such a definite effect on localization, it may be of interest to speculate about the mechanism. The heart, large blood vessels, and mediastinum of the rabbit are very flexible and shift when the animal's position changes from the normal horizontal to the abnormal vertical. This shift apparently changes the angle at which the blood from the superior and inferior vena cava enters the right auricle. An analysis of the two vertical positions and their results seems to give support to this part of the theory. In the upright position, the superior vena cava is the superior venous vessel entering the heart; the emboli localize in the superior portion of the lung, which in this position comprises the upper lobes. In the inverted position, the superior vena cava is the inferior venous vessel and the emboli localize in the inferior portion of the lung, which is again the upper lobes. In both cases, the localization occurs as would be predicted from the theory and explains why there is upper lobe localization in both of the vertical positions.

The results of the femoral vein injection do not seem to conform with the

original theory as expressed in the first part of this paper, but apparently contradict it. From the theory, it would be expected that emboli injected through the femoral vein would localize in the lower lobes as they enter the heart through the inferior vena cava. However, the original theory was formulated for man, who is predominantly an upright animal, and did not include the possibility that position would affect localization. In the experiments with the tuberculous pus, emboli injected in the femoral vein when the animal was lying on its back in the horizontal position did go predominantly to the upper lobes (table 6). But when the embolus was injected into the femoral vein and the animal was immediately shifted to the upside down vertical position, before the embolus had reached the abdominal vein, the infarcts were most numerous in the lower lobes (table 7).

The question of the quantitative influence of the comparative volume of the upper and lower lobes on the localization of emboli may be raised. As the lower lobes have a much greater volume and, hence, a greater blood supply than the upper lobes, it might be expected, as pointed out by Medlar and Sasano (3), that a majority of emboli would lodge in the lower lobe. However, when comparing the results in one group of animals which is injected in the ear vein and another in the femoral vein, or in comparing the results when the same route of injection is employed but the position of the animal is horizontal or vertical, the relative volume of upper and lower lobes remains constant. Hence, no correction is needed for the difference in volume of the lobes.

These experiments also help to explain the predominant basal localization of pulmonary emboli and infarcts (4–9). It is well known that most pulmonary emboli originate in the veins of the pelvis and legs and would, therefore, enter the heart through the inferior vena cava. In Collins' *Series 5*, the original thrombus was found in the veins which drain into the inferior vena cava in 60 per cent; in the heart, 18 per cent; and in the remaining 22 per cent, the original site of the thrombus could not be discovered.

It appears, therefore, that the localization of embolic particles in the pulmonary vascular tree is the result of the interplay of two factors: the route of entry of the particle into the right heart; and the position of the body. If, as theorized, reinfection tuberculosis in man is the result of the reactivation of an old tuberculous process and the subsequent entrance of an embolus containing tubercle bacilli into the blood stream, the location of the tuberuclous lesions is the result of these two factors. In man, the apical localization of these lesions results from the entrance of the pus embolus into the heart through the superior vena cava while man is in the upright position. However, pus from a tuberculous lesion in the lower limbs or in the abdominal cavity might enter a vein draining into the inferior vena cava and be directed by the stream flow to the lower lobes. It is assumed that man usually will be in the upright position and exerting himself when a caseous lymph node discharges its contents, but such an event might occur when man was in the supine position. Even in the latter instance, the lesion might appear in the upper lobes since man's heart, vessels, and mediastinum are not as flexible as those of the rabbit.

When the small diameter of the pulmonary artery in a rabbit is considered, it is surprising that differences between the localization in the upper lobes and lower lobes were as great as $P < 0.001$. The separation is sharper in man, in whom the diameter of the pulmonary artery is much larger. The calculation from the clinical data of Medlar (10) on reinfection pulmonary tuberculosis and from Hampton and Castleman (11) on infarcts shows that P was <0.0001.

The dog has a pulmonary artery which is intermediate in size between that of the rabbit and man. In the next paper in this series, the mechanical basis for the layering of the blood in the right side of the heart and in the pulmonary artery will be demonstrated by the injection of colored synthetic plastics.

SUMMARY

To test this theory of selective localization, *Lycopodium* spores suspended in serum were injected alternately into the ear and femoral veins of rabbits. When the animals were standing in the normal position or lying on their backs and the spores were injected into the ear veins, 76.7 to 81.6 per cent of the infarcts were found in the lower lobes and only 18.4 to 23.2 per cent were in the upper lobes. When the spores were injected into the femoral vein with the rabbits lying on their backs, the percentage of infarcts in the lower lobes was reduced to 48.1 and of those in the upper lobes increased to 51.9. This difference was found to be statistically significant.

The position of the rabbit at the time of injection also influences the localization of the infarcts. When rabbits were injected in the ear vein while in the vertical position with the head either up or down, the percentage of infarcts in the lower lobes decreased from 78.4 to 59.3 and of those in the upper lobes increased from 21.6 to 40.7.

Pus from experimental tuberculous abscesses was injected into the ear and femoral veins of alternate rabbits in the horizontal position. When the pus was injected into the ear vein, 76.75 per cent of the lesions were found in the lower lobes and only 23.25 per cent in the upper lobes. But when the same quantity was injected into the femoral vein, the lower lobe lesions were reduced from 76.75 to 46.6 per cent and those in the upper lobes increased from 23.25 to 53.4 per cent. When the tuberculous pus was injected into the femoral vein and the rabbit immediately dropped to the vertical inverted position, the number of lesions in the upper lobes decreased and the number in the lower lobes increased.

The experiments demonstrate that the location of infarcts in the rabbit's lungs can be influenced by (*1*) the position of the animal at the time of injection and (*2*) by the route of injection. The results support the theory that the blood is incompletely mixed in the right auricle and ventricle and passes through those chambers in layers.

If it be assumed that the layering effect demonstrated in rabbits is also present in man, then caseous material escaping from infected lymphatics will be transported by the lymph vessels and thoracic duct to the column of blood entering the heart through the superior vena cava. It will pass in a layer through

the right auricle and ventricle into the superior portion of the pulmonary artery and by the superior branches to the upper lobes of the lungs.

SUMARIO

La Localización Apical de la Tuberculosis Pulmonar de Reinfección: II. Localización Selectiva de los Embolos Experimentales

A fin de comprobar esta teoría de la localización selectiva, se inyectaron esporos de licopodio suspendidos en suero alternativamente en las venas auriculares y femorales de conejos. Cuando los animales estaban de pie en la posición normal o recostados sobre el dorso y se inyectaban los esporos en las venas auriculares, de 76.7 a 81.6 por ciento de los infartos radicaban en los lóbulos inferiores y solo 18.4 a 23.3 por ciento quedaban en los lóbulos superiores. Cuando se inyectaban los esporos en la vena femoral con los conejos recostados sobre el dorso, el porcentaje de infartos en los lóbulos inferiores descendía a 48.1 y en los lóbulos superiores subía a 51.9. Descubrióse que esta diferencia poseía importancia estadística.

La posición del conejo al ejecutar la inyección también afecta la localización de los infartos. Cuando se inyectó a los conejos en la vena auricular, mientras se encontraban en la posición vertical con la cabeza ya alzada o baja, el porcentaje de infartos en los lóbulos inferiores descendió de 78.4 a 59.3 y en los lóbulos superiores subió de 21.6 a 40.7.

Se inyectó pus procedente de abscesos tuberculosos experimentales alternativamente en las venas auriculares y femorales de conejos en la posición horizontal. Al inyectarlo en una vena auricular, 76.75 por ciento de las lesiones correspondieron a los lóbulos inferiores y sólo 23.25 por ciento a los superiores. Sin embargo, al inyectar la misma cantidad en una vena femoral, las lesiones de los lóbulos inferiores bajaron de 76.75 a 46.4 por ciento y las de los superiores subrieron de 23.25 a 53.4 por ciento. Al inyectar pus tuberculoso en la vena femoral y bajar el conejo en el acto a la posición invertida vertical, el número de lesiones disminuyó en los lóbulos superiores y aumentó en los inferiores.

Demuestran estos experimentos que la localización de los infartos en los pulmones del conejo puede ser afectada por: (*1*) la posición del animal al hacerse la inyección y (*2*) la vía de inyección. Los resultados apoyan la teoría de que la sangre se mezcla incompletamente en la aurícula y el ventrículo derechos y atraviesa esas cavidades en capas.

Si se da por sentado que ese efecto en capas, observado en los conejos, se presenta también en el hombre, las materias caseosas que se escapan de los linfáticos infectados serán transportadas por los vasos linfáticos y el conducto torácico a la columna de sangre que penetra en el corazón a través de la vena cava superior y, de allí, en una capa a través de la aurícula y del ventrículo derechos a la porción superior de la arteria pulmonar y por las ramas superiores de ésta a los lóbulos superiores del pulmón.

RESUME

La localisation apicale de la tuberculose pulmonaire de réinfection: II. Localisation d'élection des embolies expérimentales

Pour éprouver cette théorie de la localisation d'élection, des spores de lycopodium suspendues dans le sérum ont été injectées dans les veines auriculaires ou fémorales du lapin. Quand les animaux étaient en position normale ou couchés sur le dos et que l'injection des spores était faite dans les veines auriculaires, 76.7 à 81.6% des infarctus étaient découverts dans les lobes inférieurs et 18.4 à 23.3% seulement dans les lobes supérieurs. Quand les spores étaient injectées dans la veine fémorale, les lapins étant couchés sur le dos, le pourcentage d'infarctus dans les lobes inférieurs était réduit à 48.1 et il atteignait 51.9 pour les lobes supérieurs. Cette différence a été considérée comme avant une valeur statistique.

La position du lapin au moment de l'injection influe également sur la localisation des infarctus. Quand les lápins recevaient l'injection dans la veine de l'oreille tandis qu'ils

étaient en position verticale, tête en haut ou en bas, le pourcentage d'infarctus dans les lobes inférieurs s'abaissait de 78.4 à 59.3 et celui des lobes supérieurs s'élevait de 21.6 à 40.7.

Le pus provenant d'abcès tuberculeux expérimentaux était injecté dans les veines auriculaires et fémorales chez des lapins alternés placés en position horizontale. L'injection du pus dans la veine de l'oreille a permis d'observer 76,75% des lésions dans les lobes inférieurs et 23,25% seulement dans les lobes supérieurs. Toutefois, quand l'injection d'une même quantité était partiquée dans la veine fémorale, les lésions du lobe inférieur étaient réduites de 76,5% à 46,6% et celles du lobe supérieur augmentaient de 23,25% à 53,4%. Quand le pus tuberculeux était injecté dans la veine fémorale et que le lapin était immédiatement mis en position verticale inversée, le nombre des lésions dans les lobes supérieurs s'abaissait et celui des lésions des lobes inférieurs augmentait.

L'expérimentation démontre que la localisation des infarctus dans les poumons du lapin peut être influencé par 1° la position de l'animal et 2° par la voie utilisée pour l'injection. Les résultats viennent à l'appui de la théorie du mélange incomplet du sang dans l'oreillette et le ventricule droit, le sang passant dans ces cavités par couches successives.

Supposant que ce "layering effect" démontré chez le lapin est également présent chez l'homme, la matière caséeuse issue des lymphatiques infectés serait donc transportée par les vaisseaux lymphatiques et le canal thoracique jusqu'à la colonne sanguine pénétrant dans le cœur par la veine cave supérieure, d'où elle passe par couches successives à travers l'oreillette et le ventricule droit dans la partie supérieure de l'artère pulmonaire et par les branches supérieures de celle-ci dans les lobes supérieures du poumon.

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THE APICAL LOCALIZATION OF REINFECTION PULMONARY TUBERCULOSIS[1]

III. The Mechanical Basis for the Layering of Blood in the Right Heart and Pulmonary Artery as Revealed by the Injection of Synthetic Plastics

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INTRODUCTION

The various theories proposed to explain the apical localization of reinfection pulmonary tuberculosis have been discussed and a new one added (1). The new theory is supported by the selective localization of experimental emboli in the lungs of rabbits (2).

This new theory, which has been designated the "stream flow" theory, is based on the simple hydrodynamic principle that fluids of the same viscosity may flow side by side through tubes for a considerable distance with a minimum of admixture. It is assumed that the blood from the superior vena cava and that from the inferior vena cava do not have time to be thoroughly mixed in the right auricle and right ventricle. Instead, the blood from the two channels passes through these chambers in layers in such a manner that most of the blood from the superior vena cava goes out in the superior portion of the pulmonary artery and, hence, by its superior branches to the upper lobes. The source of the reinfection or reactivation tuberculosis is frequently a caseous lymph node which discharges its contents into the lymphatic vessels which, in turn, drain into the thoracic duct and, hence, to the left innominate vein and on into the superior vena cava. This also explains the predominant localization of pulmonary infarcts in the lower lobes as the embolic material usually originates in the veins of the legs and pelvis and reaches the heart through the inferior vena cava and is directed by the "stream flow" into the lower lobes.

In the present study an attempt was made to demonstrate the purely mechanical aspects of the "stream flow" theory by injecting colored plastic material into the right auricle of dogs through the superior and inferior venae cavae. The colors used were red and green, one being injected into the superior and one into the inferior vena cava. The plastic solutions of equal viscosity were injected simultaneously through tubes of the same length and diameter and under the same head of pressure to eliminate mechanical factors other than the hypothetical layering of the two streams in the right auricle, right ventricle, and pulmonary artery (figure 1).

MATERIALS AND METHODS

The technique used was a modification of the one employed by Liebow and associates to obtain plastic casts of the pulmonary and bronchial vessels (3). The plastic, Vinylite

[1] From the Department of Bacteriology, School of Medicine, Duke University, Durham, North Carolina.

Specification 1,[2] was dissolved in acetone to make a 28 per cent solution and colored by the addition of either a red or a green acetone-soluble dye. Calco Oil Green 0–9660 special and Calco Oil Red N–1700 were employed.[3] The powdered dye was added to the plastic in sufficient amount to produce a clear, intense color.

It was found that careful preparation of the anatomic specimen was essential for success. Dogs were narcotized with Nembutal,® after which the abdomen was opened and the aorta severed. The inferior vena cava was ligated just above the diaphragm and cannulated with a glass tube 8 mm. in diameter. After cannulation, 100 to 155 cc. of 2.5 per cent sodium citrate were injected to flush out the right auricle, right ventricle, and pulmonary artery. The superior vena cava was ligated proximal to the entrance of the subclavian vein and cannulated. Heart action usually ceases during this procedure. The glass tubes were moved into the right auricle until they met in the center of this chamber, after which they were withdrawn until the ends approximated the caval orifices. The free ends of the tubing were connected to the fluid reservoirs, and 200 cc. of acetone were flushed through the system to remove the free water. This was found to be a necessary step because preliminary experiments indicated that a few drops of water produced violent convection currents in the acetone-plastic solution. Following the injection of the acetone, compressed air at approximately 22 pounds of pressure was forced through the heart and vessels to eliminate all acetone and dry the vascular bed. This step was necessary because acetone in varying concentrations also induced convection currents. Finally, 100 to 200 cc. of plastic were injected under a pressure of 22 pounds. The entire amount of plastic was injected in thirty to forty seconds. Several preparations were made with red plastic injected into the superior vena cava and green in the inferior vena cava, while in other preparations the colors were reversed.

In the first series of experiments, the dogs were lying on their backs in the horizontal position when the injections were made. In the second series, the dogs were strapped to an operating table and held in a vertical position during both the preliminary perfusion and the subsequent injection of plastic.

Following the injection, the venae cavae were ligated, the cannuli removed, and, without altering the position, the animal was carefully placed in the ice box for twelve to twenty-four hours. Subsequently, the lungs and heart were removed and all tissue digested by immersion in hydrochloric acid. This process required twenty-four to forty-eight hours. The lungs were removed from acid as soon as digestion was effected because prolonged exposure to the acid tended to fade the color. The specimen was defatted by immersion in petroleum ether for four to six hours.

OBSERVATIONS

Experiment I

In three experiments, the lung-heart preparations were injected with the green plastic through the superior vena cava and the red plastic through the inferior vena cava. The dogs were in a supine position during the entire injection.

In each case the red plastic predominated in the vessels of the upper lobes. When found in middle or lower lobes, it was seen to be filling branches arising from posterior aspects of the pulmonary arteries. The basal portions were uniformly green, with the line of division between red and green varying somewhat

[2] The writers are indebted to Mr. J. C. Fryman, Bakelite Company, Division of Union Carbon and Carbide Chemicals Corporation, Thermoplastics Dept., New York 17, N. Y., for providing the Vinylite used in the studies.

[3] These materials were kindly provided by Calco Chemical Division, American Cyanamid Company, Dyestuffs Division, Bound Brook, New Jersey.

but usually lying in the right middle lobe or superior proportions of the left lower lobe.

Although equal volumes of the two colors were injected, it was noted that there was uniformly a preponderance of green. It was assumed that there was some mixing of the colors at the interface with the deeper green masking considerable quantities of the red. This assumption was confirmed by mixing varying proportions of the colored plastic in test tubes. To clarify this problem, the colors were reversed so that green plastic was injected into the inferior vena cava and red into the superior vena cava. Seven dogs were injected in this manner. The results were essentially confirmatory, with the red plastic from the superior vena cava predominating in the basal rami of the pulmonary artery while the apical branches contained the green plastic from the inferior vena cava.

Experiment II

In the second set of experiments, the dogs were held in the vertical position. Cannulation and perfusion were accomplished exactly as in the previous experiment. Red plastic was injected into the superior vena cava and green into the inferior vein.

Eight dogs were perfused in this manner. Two of these preparations were technically unsatisfactory. One specimen presented essentially the same distribution of the plastic as that seen in the previous experiment.

The remaining five preparations evidenced a reversal in the distribution of the plastic as compared with that in the supine animal. Thus, in the vertically placed animal, the red Vinylite entering the superior vena cava was preferentially distributed to the apical branches of the pulmonary artery and to some extent to middle lobe branches arising on the posterior aspect of the artery. The green Vinylite from the inferior vena cava was chiefly confined to the lower lobes.

Experiment III

In the third series of experiments, the dogs were fixed in the vertical position, after which both subclavian veins and the inferior vena cava were cannulated. Plastic solution colored red was injected into the inferior vena cava and the right subclavian vein. A green-colored plastic was injected into the left subclavian vein. The injections were made with a modification of the apparatus shown in figure 1. The colored plastics were released in the three vessels simultaneously. Some difficulty was encountered in releasing the three columns of plastic at the same instant, but when this was achieved the layering effect was demonstrated as shown in figure 2*A*.

The green-colored plastic, which was injected into the left subclavian vein, met the red-colored plastic from the right subclavian vein and continued into the superior vena cava. The green-colored plastic, from the left subclavian vein, was distributed to both upper lobes and was the predominant color. Never-

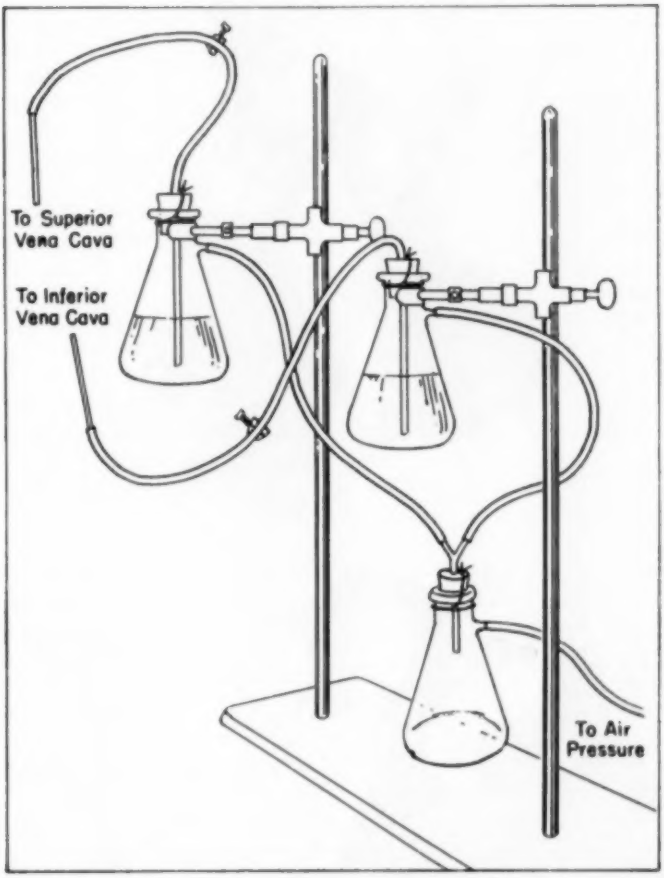

FIG. 1. Perfusion apparatus. In this perspective drawing, the two upper flasks, maintained at the same level, serve as fluid reservoirs in which the pressures are equalized by the lower flask containing only air. The fluid reservoirs are filled successively with the various perfusates, the system closed, and perfusion is accomplished by pressure.

theless, there were a number of small areas which were pure red, indicating that even under these conditions the mixing of the solutions, as they passed through the right heart, was not complete.

The delicacy of this layering mechanism is illustrated in figure 2*C*, in which both a green and red column can be seen in the artery supplying a segment of a lobe. As branching occurred, pure green appeared in the lung on one side of the vessel and pure red on the other.

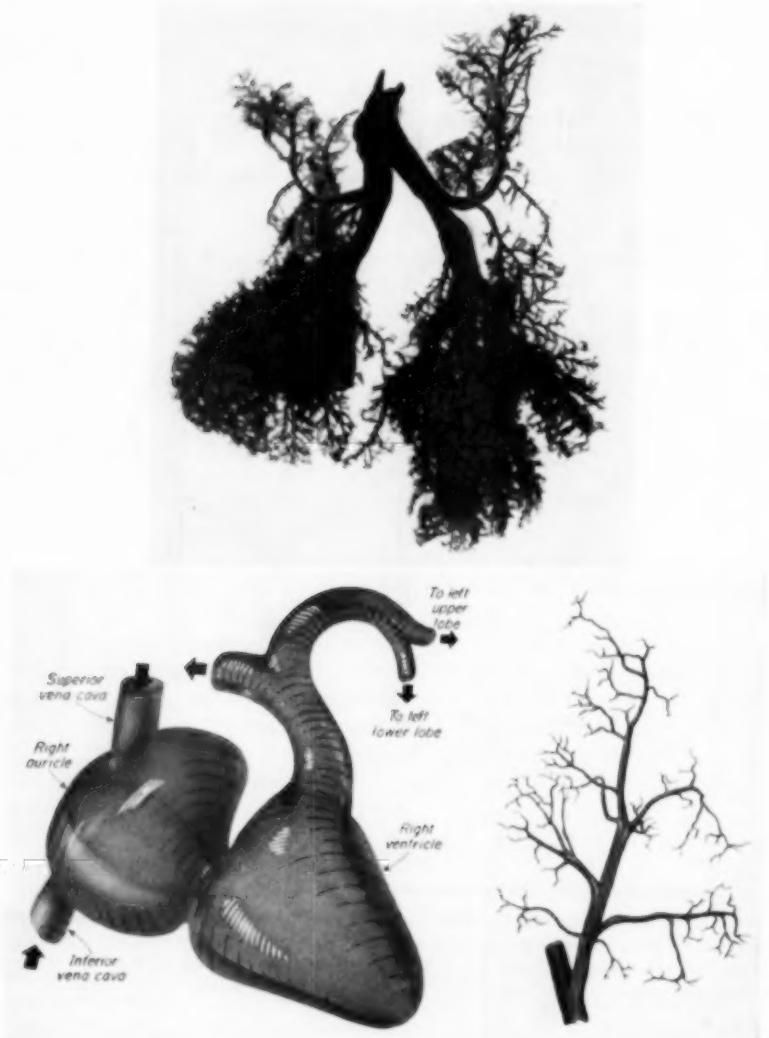

FIG. 2. *A*. Kodachrome photograph of Vinylite model of pulmonary arterial tree. The dog was in the vertical, upright position when the plastic was injected into the left subclavian vein and red-colored plastic in the right subclavian and inferior vena cava. The green plastic went to both upper lobes but some small areas received the red plastic from the left subclavian. The lower lobes are red from the red plastic injected into the inferior vena cava. *B*. This drawing shows the layering of the colored plastics as they pass through the right side of the heart and into the pulmonary artery. *C*. The delicacy of the layering mechanism is illustrated in this drawing of segmental branches. Note the layering of the green and red in the main artery with green branches on one side and red on the other.

DISCUSSION

An attempt to ascertain the course of the two columns of plastic through the right heart is made more difficult by the tendency for mixing to occur in the chambers of the heart after the plastic has ceased to flow and before it hardens. As a result of this mixing, the plastic within the heart is usually of a neutral shade. Occasionally, however, the mixing is incomplete and in such preparations one finds a uniformity in the location of the colors sufficient to form the basis of a description of the course of the two columns.

It seems that the principal change involved in the postural reversal of the distribution of the plastics is a change in the angle of entry of the inferior vena cava. When the animal is moved from the supine to the vertical position, the diaphragmatic portion of this vessel moves anteriorly, causing the stream of flow to be in a more posterior direction. Thus, with the animal vertically disposed, the contents of the superior vena cava come to occupy an anterior position in the right atrium as seen in several of the specimens. Apparently, the plane of the interface between the two columns is not altered during the flow into the ventricle, for here, too, the plastic from the superior vena cava is seen to occupy an anterior position. As the pulmonary trunk turns posteriorly, this plastic comes to lie first in the superior portions of the trunk and then, as the pulmonary arteries course caudally, the plastic which was originally introduced through the superior vena cava occupies a posterior position (figure 2*B*) to form a posterior column. As the upper lobe branches uniformly arise from the posterolateral aspects of the pulmonary arteries, it is into these branches that this posterior column flows.

SUMMARY

The experiments presented support the "stream flow" theory for the apical localization of reinfection tuberculosis. There are mechanical factors in the cardiovascular system of the dog which direct the flow of the plastic entering the right auricle to a specific location in the lung. When the dog is in the supine position, the plastic entering the superior vena cava is directed largely to the lower lobes. But when the dog is in the vertical position, corresponding to the upright position of man, the plastic entering the superior vena cava is directed predominantly to the upper lobes.

The distribution of the green-colored plastic to both upper lobes, when it was injected into the left subclavian vein, suggests that caseous material which reaches the left subclavian from the thoracic duct might localize in either the right upper, left upper, or both upper lobes.

SUMARIO

La Localización Apical de la Tuberculosis Pulmonar de Reinfección: III. La Base Mecánica para la Formación de Capas por la Sangre en el Corazón Derecho y la Arteria Pulmonar Según Reveló la Inyección de Substancias Plásticas Sintéticas

Estos experimentos apoyan la teoría que imputa al flujo de la corriente de sangre la localización apical de la tuberculosis de reinfección. En el aparato cardiovascular del

perro existen factores mecánicos que encaminan el flujo de la substancia plástica que penetra en la aurícula derecha a un sitio específico del pulmón. Cuando el perro se encuentra en la posición supina, la substancia que penetra en la vena cava superior es en gran parte encaminada a los lóbulos inferiores. Sin embargo, cuando el perro toma la posición vertical, que corresponde a la erecta en el hombre, la substancia plástica que penetra en la vena cava superior es encaminada predominantemente a los lóbulos superiores.

La distribución de la substancia plástica teñida de verde a ambos lóbulos superiores, cuando se inyectó en la vena subclavia izquierda, sugiere que las materias caseosas que llegan a dicha vena por el conducto torácico podrían localizarse ya en los lóbulos superiores derecho o izquierdo o en ambos.

RESUME

La localisation apicale de la tuberculose pulmonaire de réinfection: III. Base mécanique du "layering effect" (déposition en couches) du sang dans le coeur droit et l'artère pulmonaire révélée par l'injection de plastique de synthèse

Ces expériences confirment la théorie du courant sanguin comme facteur de la localisation apicale de la tuberculose de réinfection. Il y a dans le système cardio-vasculaire du chien des facteurs mécaniques qui font qu'une matière plastique pénétrant dans l'oreillette droite est dirigé par le courant sanguin vers une partie spécifique du poumon. Quand le chien est en position de supination, le plastique qui entre dans la veine cave est principalement dirigé vers les lobes supérieurs.

La distribution du plastique coloré en vert dans les deux lobes supérieurs, lorsqu'il est injecté dans la veine sous clavière gauche, suggère que la matière caséeuse qui atteint la sous-clavière gauche à partir du canal thoracique peut se localiser soit dans le lobe supérieur droit ou gauche, ou dans les deux lobes supérieurs.

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THE EFFECTS OF ISONIAZID ON PATIENTS WITH MULTIPLE SCLEROSIS[1]

Preliminary Report

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INTRODUCTION

Multiple sclerosis is a common disease of unknown etiology, variable course, and undefinable prognosis for the individual (1). The evaluation of therapy in this disease is fraught with many difficulties because attacks or exacerbations may last for varying periods, and the completeness of remission is unpredictable. With due recognition of these many difficulties, it is believed appropriate at this time to report certain observations on the course of multiple sclerosis during the administration of isoniazid. The observations were made on a group of patients, hospitalized for multiple sclerosis in the past eighteen months, who were given isoniazid. This group of patients was compared with a similar group of persons hospitalized for multiple sclerosis in the last ten years. Individuals of both groups were classified on admission and discharge in accordance with a rating scale devised by the writers. The results of this comparison are presented to show the apparent influence of isoniazid on manifestations of multiple sclerosis.

As an agent for study in multiple sclerosis, isoniazid was suggested accidentally when a chronically ill patient, who had received isoniazid for another purpose, was noted to speak more distinctly. The drug was then tried sporadically in various dosages on a few other patients and, when there seemed to be some effect, a formal program was instituted.

PLAN OF INVESTIGATION

In the present study, the patients given isoniazid[2] were compared with a group of 175 patients with multiple sclerosis who had been admitted to this hospital from 1944 to 1953, inclusive. Patients admitted between 1944 and 1950 were classified on the basis of the hospital records, and all patients admitted after 1950 were evaluated by one or both of the writers on admission and discharge, in addition to the evaluations by the patients' own staff physicians. The percentage of patients in the comparison group who improved during these years of personal observation by the writers (31 per cent) was the same as the percentage of previous patients (34 per cent) whose improvement had been recorded in the hospital records.

An additional 45 patients with multiple sclerosis were not included in the comparison group because the status of their multiple sclerosis had not changed within the two-year period preceding admission to the hospital. Of this group,

[1] From the Neurology Section, Veterans Administration Hospital, Bronx, New York.

[2] Supplied as Rimifon by Hoffman La Roche, Inc., Nutley, New Jersey.

none improved and 5 became worse. (In the writers' experience, two years represents the *maximal* period a particular clinical manifestation of multiple sclerosis may persist and there yet be some possibility of significant spontaneous improvement).

Isoniazid, 300 mg. daily in divided doses, was given to patients with multiple sclerosis throughout their hospitalization. "Maintenance" therapy of 200 mg. per day has been given to a number of these patients after discharge from the hospital. Most of the patients were unaware of their diagnosis until discharge from the hospital. None were told prior to discharge that they were receiving any special medication. Suggestion was minimal. Therapy otherwise was in accordance with previous standards at this hospital, including physical medicine and rehabilitation procedures; the only exception was that no other medication was given. Even supplementary vitamins were not given to the patients who received isoniazid.

The duration of hospitalization was determined by the progress of the patient's illness. A period of two to four weeks of stabilization of the illness was required before a patient was discharged as having attained maximal hospital benefit. As this is not a chronic-disease hospital, no exceedingly prolonged periods of hospitalization were encountered in any of the groups.

Classification of Status

In order to evaluate the course in this protean disease, the classification or status of an individual patient was made in accordance with a scale devised by the writers in such a way that, first, it would be applicable to all patients and, second, that it would represent the sum of neurologic dysfunction.

The usual course of multiple sclerosis results in greater incapacity of the legs than of the arms. The usual involvement of head segment functions, in the absence of pyramidal disorder, is a minor disability. The scale used, with categories from 0 to 10, is one of a progression (not necessarily arithmetical) with different levels of functional ability in each category:

0—normal neurologic examination
1—minimal signs, no symptoms or incapacity (extensor plantar response, minimal finger-to-nose ataxia, diminished vibration sense)
2—minimal symptoms (slight weakness or stiffness, mild disturbance of gait, awkwardness, mild visuomotor disturbance)
3—moderate symptoms (monoparesis, mild hemiparesis, moderate ataxia, disturbing sensory loss, prominent urinary or eye symptoms, or combinations of lesser dysfunctions)
4—relatively severe symptoms not preventing ability to work or carry on normal activities of living, excluding sexual functions
5—symptoms severe enough to preclude working, with maximal motor function permitting walking unaided up to several blocks
6—assistance required for walking (canes, crutches, braces)
7—restricted to wheelchair (able to maneuver chair and enter and leave chair without aid)
8—restricted to bed but with effective use of arms
9—totally helpless bed-patient
10—death due to multiple sclerosis

Upon the basis of the records of 300 cases of multiple sclerosis and the reports in the literature, the occurrence of significant dysfunction which is not suitably assignable in this classification is extremely rare. It may be seen that "incapacitation" (inability to walk unaided) includes categories from 6 on. While this grouping is not perfect, it provides a means of evaluating all of the 250 cases.

Improvement, as defined in the present report, means a gain of at least one category between admission to and discharge from the hospital.

Composition of the Two Groups

The isoniazid group consisted of 30 white male veterans. The comparison group contained but one female (0.6 per cent) and 6 Negroes (3.5 per cent); the remainder were white male veterans.

The two groups included only those patients whose illness was characterized by an exacerbation within two years of admission except for one patient (No. 29) in the isoniazid group, whose present episode was of three years' duration.

The two groups were essentially identical in age at the time of admission, age at onset of disease, and duration of the present exacerbation before admission to the hospital. There was no significant difference between the groups with respect to length of time in the hospital and total duration of illness from onset of first symptoms. The mean age on admission to the hospital was 32.1 years for the comparison group and 33.2 years for the isoniazid group. The mean age at the time of onset of the multiple sclerosis was 27.6 years (comparison) and 27.2 years (isoniazid). The mean duration of the present exacerbation or episode to admission was 182 days (comparison) and 174 days (isoniazid), respectively. The mean total duration of illness in the comparison group was fifty-one months, with a mean of seventy-one months for the isoniazid patients. The mean length of hospital stay was one hundred and four days (comparison) and seventy-nine days (isoniazid).

OBSERVATIONS

Course of illness in comparison group: In the comparison group of 175 patients, improvement (or remission) occurred in 58 patients (33 per cent), with an average gain of two categories between admission and discharge. The comparison group also contained 88 patients (50 per cent) whose status remained the same and 29 (17 per cent) whose illness became worse (with an average loss of two categories) during hospitalization.

On admission to the hospital there were 21 members (12 per cent) of the comparison group with minimal or no symptoms (category 2, or less), and on discharge 48 patients (27 per cent) were of such status. Fifty-eight patients, or one-third of the comparison group, were in category 6 or higher, both on admission and on discharge. In categories 3 through 5 (moderate impairment), there were 96 patients (55 per cent) on admission and 69 patients (39 per cent) on discharge.

Of the 21 patients admitted in category 2 or less, 6 improved and one worsened while in the hospital. Of the 96 patients admitted in categories 3 through 5, 34

(35 per cent) improved and 14 (15 per cent) worsened; of the 58 patients in category 6 or higher on admission, 18 (31 per cent) improved and 14 (24 per cent) became worse during hospitalization. The remainder in each subgroup experienced no change in status between admission and discharge.

Course of illness in isoniazid group: In the group of 30 patients who received isoniazid, 27 (90 per cent) showed improvement in the status of their multiple sclerosis by an average of three categories. In 2 patients (7 per cent), the disease remained unchanged and, in one, it became worse. The one patient whose disease worsened was a man (patient No. 29) admitted with disease of category 9 who, without change in neurologic status, died of bronchopneumonia and hemorrhagic cystitis. The outcome of this patient is classified as a failure even though his present episode was a progressive one of three years' duration. Of the other 2 patients who did not improve (patients Nos. 17 and 30), one had had unchanged manifestations of multiple sclerosis for almost two years before admission; the other had first experienced his present episode only six months previous to admission.

Only one member of the isoniazid group had minimal symptoms at the time of admission to the hospital, whereas 15 of the 30 isoniazid patients had minimal or no symptoms when discharged from the hospital.

Inability to walk unaided (category 6 and higher) was present in 19 of the 30 isoniazid patients on admission and was still present in 10 at the time of discharge.

Moderate involvement (categories 3 through 5) was present on admission in 10 patients and was still present in 5 patients on discharge.

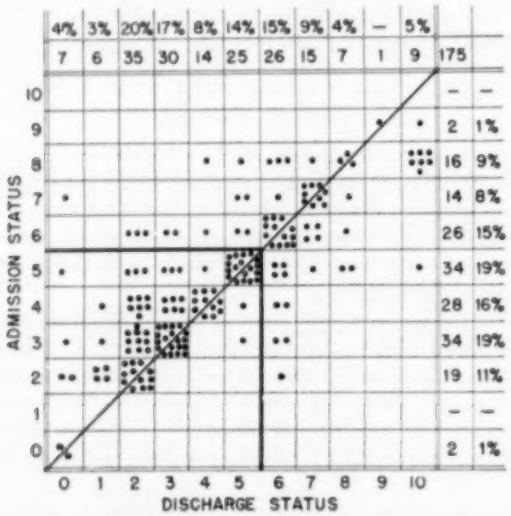

FIG. 1. Comparison group "A". Course in hospital of patients with multiple sclerosis whose present episode is two years or less.

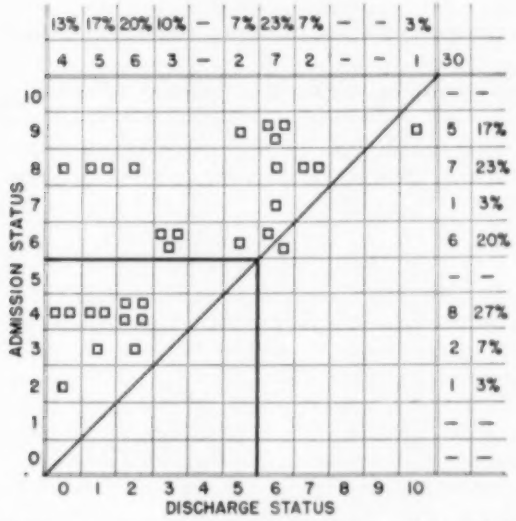

FIG. 2. Isoniazid group. Course in hospital of patients with multiple sclerosis.

FIGS. 1 and 2. These figures graphically present the admission and discharge status of each patient with multiple sclerosis. The abscissa represents the discharge status in accordance with the categories of involvement devised by the writers and presented in the body of the paper. The ordinate similarly represents admission status. The larger the status number, the more severe the impairment. The boxes bisected by the diagonal line represent no change in status between admission and discharge. In figure 1, each patient is represented by a dot; in figure 2, each patient is depicted as a small square. The localization of each dot or square depends on admission and discharge status. For example, in figure 2, there are 3 squares in the box which is along "6" on the ordinate (admission) and "3" on the abscissa (discharge); this represents three patients admitted as category 6 and discharged as category 3, i.e., these patients gained 3 categories in the hospital.

More patients who received isoniazid improved in each sub-class than did those in the equivalent sub-class of the comparison group.[3] The percentage of

[3] Statistical methods were used to compare the percentage of patients who improved in the hospital in the comparison group (33 per cent) with the percentage who improved in the isoniazid group (90 per cent). The results showed that there was a highly significant difference well beyond the 0.1 per cent level.

Chi-square test using Yate's correction for fourfold contingency tables (with one degree of freedom):

$$\chi_c^2 = \frac{(|ad - bc| - N/2)^2 N}{(a + b)(c + d)(a + c)(b + d)} = 31.8$$

$P = 0.001$ for a χ_c^2 of 10.83.

a = 58 = number of "successes" in comparison group
b = 27 = number of "successes" in isoniazid group
c = 117 = number of "failures" in comparison group
d = 3 = number of "failures" in isoniazid group
N = 205 = a + b + c + d

TABLE 1

ISONIAZID IN MULTIPLE SCLEROSIS: SUMMARY OF 30 CASES

Case Number	Days in Hospital	Age at Admission	Days Present Episode	Months Total Duration	Age at Onset	Status	
						Admission	Discharge
1	110	33	21	36	30	8	1
2	17	39	14	0.5	39	4	0
3	63	38	16	120	28	4	1
4	77	35	730	21	33	4	2
5	53	31	90	5	31	4	2
6	24	27	42	12	26	4	2
7	210	31	30	37	27	9	6
8	42	56	450	204	39	6	3
9	42	25	180	72	19	3	2
10	11	31	2	2	31	3	1
11	79	25	21	24	23	4	1
12	93	33	21	108	24	6	3
13	150	38	730	144	26	9	6
14	21	29	6	0.2	29	8	0
15	30	44	42	108	35	4	0
16	150	40	27	1	40	9	6
17	60	35	730	72	29	6	6
18	106	43	180	144	31	9	5
19	36	29	240	156	16	6	3
20	106	30	90	74	24	8	6
21	97	24	35	1	24	8	1
22	42	28	30	84	21	7	6
23	34	27	5	72	19	8	2
24	21	24	14	24	22	4	2
25	16	29	7	73	23	2	0
26	179	34	11	100	26	6	5
27	134	34	180	90	27	8	7
28	140	32	5	132	21	8	7
29	120	43	1095	120	33	9	10
30	114	28	180	96	20	6	6

improvement or worsening was not markedly different in all three sub-classes (minimal, moderate, and severe) from the percentages for the total group of 175 patients, and the same was true for the isoniazid series of 30 when compared with its own sub-classes.

As noted above by the system of rating used, the isoniazid group was, in general, a more severely ill group than the comparison series at the time of admission to the hospital. Despite this, in the isoniazid group the average gain was three categories in comparison with an average gain of two categories among those who improved in the comparison series. A comparison was made of the discharge distribution among the various categories of the isonizaid group with the discharge distribution of the comparison group. It should be noted that the

latter group was weighted against the isoniazid group in this respect because of the larger admission percentage of less severely involved patients. Nevertheless, the distribution of the isoniazid group at discharge showed a higher proportion of patients with less severe disability, and this difference between the two groups was significant at the 5 per cent level.[4]

All of the above data are presented graphically in figures 1 and 2 and, in addition, a summary of the pertinent information concerning the 30 patients in the isoniazid group is presented in table 1.

Relation of Isoniazid Administration to Remission or Relapse

The course of the manifestations of illness in 4 of the patients who received isoniazid is of particular interest with respect to the question of an influence of isoniazid on the course of multiple sclerosis. Isoniazid was administered to each of these patients (Nos. 1, 4, 7, and 12) and they promptly showed improvement of their illness. The drug was then stopped and an exacerbation of the illness occurred. Isoniazid was reinstituted and, concomitant with this, improvement again occurred in each of these patients, and in each case resulted in a status superior to that present on admission. Brief case reports of these patients follow:

Case 1. The patient, I. B., 33 years of age, was admitted to the hospital on April 8, 1953, with left-sided weakness and numbness for three weeks. Four months and also two years before admission, he had had episodes of paresthesias of the right leg and weakness of both arms and right leg, each lasting several weeks. Examination showed marked scanning of speech, nystagmus, poor convergence, diplopia on left lateral gaze, and skew deviation on inferior gaze. There was marked left hemiparesis, hemihypalgesia, and hemihypesthesia. The cerebrospinal fluid protein was 55 mg. per 100 ml.

Isoniazid (200 mg. daily) was started on April 18; marked improvement in power, sensation, and eye movements followed. The drug was stopped on April 21, with a subsequent exacerbation, so that by April 28 the patient was unable to stand. He had a marked quadriparesis, ataxia of the arms, decreased position and vibration sense in the legs, and bilateral extensor plantar responses.

Isoniazid (200 mg. daily) was again given from May 8 to 22, with immediate reversal of the course of illness. From May 27 to discharge on August 10, 1953, the patient received isoniazid, 150 mg. daily. By June 19, head functions were normal except for minimal blurring of vision, coordination was normal, gait but slightly ataxic, power was good, sensation

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[4] Chi-square test for twofold distribution (Fisher):

$$\chi^2 = \frac{\Sigma(Ap) - p\,\Sigma(A)}{\bar{p}\,\bar{q}} = \frac{6.855 - 0.146 \times 30}{0.146 \times 0.854} = 19.82$$

for $n = 10$, $P = 0.05$ at $\chi^2 = 18.31$

n = degrees of freedom = (rows -1) (columns -1) = 10

A' = number of isoniazid patients in any row, e.g., 4 in category 0

$p' = A'/t'$; $t' = A'$ + number of comparison patients in same row, e.g. 11 in category 0

$\bar{p} = \Sigma(A)/N = 30/205 = 0.146$

$\bar{q} = \Sigma$(comparison group)$/N = 175/205 = 0.854$

N = total number of cases

(*See* figures 1 and 2 for the numbers in each A subgroup.)

normal, reflexes physiologic, and once again he was potent sexually. He remained in this status until the present time, sixteen months after the onset.

Case 4. F. G., a 36-year-old man, was admitted to the hospital on October 19, 1953, with progressive dysarthria, ataxia, and stiff legs for two years. Examination revealed unilateral nystagmus and left external rectus palsy. His speech was dysarthric and slightly scanning. There was marked limb ataxia and moderate gait ataxia with pyramidal tract signs in all of the four extremities and weakness of the left foot, with decreased position and vibration in the legs and dysstereognosis of the left hand. The cerebrospinal fluid had a *D* curve with 49 mg. of protein per 100 ml. and a gamma globulin of 9.2 mg. per 100 ml.

There was no change in the patient's status until October 28, when isoniazid (300 mg. daily) was started. In three days his gait improved. By November 17, the plantar response was normal; and power, gait, sensation, and coordination were normal. The left sixth-nerve palsy persisted. Isoniazid was stopped on November 20 and within three days progressive dysfunction reappeared with mild dysarthria, ataxia of gait and limbs, and fatigue. From December 1, 1953, to discharge on January 6, 1954, isoniazid (300 mg. daily) was again given, with the return of improvement. On discharge there was persistence of the left sixth weakness without diplopia and minimal difficulty in heel-to-toe walking with eyes closed. At present the patient is asymptomatic.

Case 7. F. K., a 31-year-old man, was readmitted to the hospital on July 8, 1953, because of multiple sclerosis which had first been noted in 1950. For his third episode he had been admitted for one month in 1952 without change, at which time he had marked ataxia, nystagmus, decreased sensation in the arms, and pyramidal tract signs. Since that time the course of the illness had been generally progressive and for one month before the present admission he had been bedridden. Examination on admission to the hospital showed triplegia with ataxia of the remaining limb, scanning speech, nystagmus, diplopia, and diminished sensation below the head. From July 9 to 22, he received isoniazid (200 mg. daily) with "50 per cent improvement." By August 5, two weeks after cessation of isoniazid, he could walk (spastic-ataxic) with canes, and arm strength was fairly good. On August 7, however, he developed urinary incontinence, blurred vision, and diplopia. Cystometry showed spastic neurogenic bladder. Within a week these new symptoms partially abated, and the course was then stable. Isoniazid, 300 mg. daily, was begun again on October 29 and the gait improved. By January 10, 1954, speech had returned to normal, there was no diplopia or nystagmus, and arm strength was good. The gait was still spastic-ataxic but cane-walking was well performed and unaided ambulation was managed for short distances. Improvement has been maintained until the present date.

Case 12. L. P., a 33-year-old man, was admitted to the hospital on August 4, 1953. Nine years before he had had an episode of ataxia, with another bout two years later. For the two years before admission to the hospital he had difficulty maintaining balance, clumsiness of the hands, intermittent diplopia, urinary urgency, and inability to maintain erections. Three weeks before admission, progressive weakness of the right leg began. Dissociation of eye movements, uniocular and bilateral nystagmus, and temporal pallor were observed. Extremely severe limb and truncal ataxia plus severe spastic right leg weakness and decreased position and vibration sense in the legs were noted. Isoniazid (100 mg. daily) was given from August 7 to September 4, with increased power of the right leg. Upon cessation of the drug, his status immediately began to worsen so that by September 14 he was severely quadriparetic and bedridden, with diplopia, more nystagmus, and even greater ataxia. Isoniazid (100 mg. daily) was resumed on September 14. By September 21 the patient could walk without loss of balance. On discharge on October 16, 1953, there was still some urinary urgency, mild ataxia and nystagmus, decreased vibration of the feet, and mild weakness of the right leg; but all other functions were normal.

These 4 patients with typical multiple sclerosis had an apparent response to isoniazid on two separate occasions during the same period of hospitalization, with a recrudescence of symptoms when administration of the drug was halted. With only 3 other patients was the drug stopped before discharge, and these men maintained their improved status.

DISCUSSION

Thirty patients with multiple sclerosis were given isoniazid while in the hospital and all but 3 showed significant improvement in the status of their disease, i.e., a gain of one or more of the ten rating categories of involvement described above. Four of these patients had exacerbation of illness with cessation of the drug and improvement on again receiving isoniazid. The course of illness of these 30 patients was compared with that of 175 patients with multiple sclerosis who were equivalent in age, sex, duration of present exacerbation, duration of total illness, and length of hospitalization. Of this comparison group, 33 per cent had showed improvement of their disease and in 17 per cent it had become worse.

It is realized that evaluation of "therapy" in multiple sclerosis is extremely difficult. Previously it was considered impossible to evaluate a drug in less than one year's time, with five years being preferred. MacLean and Berkson (2) state that an effective therapy should result in "stabilization (or improvement) of symptoms in at least 96 of 100 patients followed for one year." Compston (3) states "the essential criterion of efficacy of any form of therapy must be its ability to prevent relapses," and believes that five years is necessary for evaluation.

The present writers believe that by the use of large series and well-defined criteria the course of multiple sclerosis observed under standardized hospital conditions should permit evaluations of therapy. The comparison group used in the present study is considered to be representative of multiple sclerosis as a whole. The finding that one-third of the comparison group were "incapacitated" (unable to walk unaided) fifty-one months after onset is in accord with the findings of MacLean and Berkson (2). They evaluated more than 400 cases and reported that one-third were incapacitated seven years after onset and two-thirds were incapacitated (unable to walk or work) five years later. Muller (4) found that 40 per cent of 810 patients were incapacitated within five years after onset.

The fact that one-third of the comparison group of the present study experienced improvement while in the hospital is in agreement with the results reported for patients under various forms of management (5, 6). Of Merritt's (5) group of 33 patients with multiple sclerosis who were treated with corticotropin, one-third showed improvement during hospitalization, and the illness of the remainder stayed essentially unchanged. Improvement of multiple sclerosis in one-third to one-half of the patients during various other forms of therapeutic management has been reported by a number of investigators (6) and may be

taken as a fair index of the expected course in the hospital of groups of patients with multiple sclerosis.

It is realized that 30 cases is indeed a small number from which to draw inferences in a disease so difficult to evaluate as multiple sclerosis. Nevertheless, the fact that measurable improvement occurred in such a high proportion of consecutively treated patients seems impressive.

The use of the chi-square test mitigates this objection to the size of the treated series to some extent, as it, of necessity, incorporates the numbers used. Although this statistical test cannot be used as a basis for evaluating the efficacy of a drug, the results of this test indicate that the outcome in the two groups was significantly different. The only differences *of which the present writers are aware* are the administration of isoniazid and the fact that the comparison group and the isoniazid group were not studied concurrently. With respect to the latter difference, it should be noted that all members of the comparison group who were admitted to the service during the three years immediately preceding the isoniazid study were examined by one or both of the present writers. All factors which could be equated were so handled, and the two groups were comparable in sex, age, and duration of illness.

Because there may be hidden differences, in the present study it cannot be stated with certainty that isoniazid is an effective treatment in multiple sclerosis. The only way in which the question can definitely be settled is by study of concurrently observed patients with comparable illnesses whose therapy with either isoniazid or a placebo is determined solely by a chance selection method. At present the inferences from the present study are in the *post hoc ergo propter hoc* class. In the 4 cases presented in detail, the fact that a temporal relationship of isoniazid to improvement appeared to exist on two separate occasions during the same hospital stay certainly seemed highly suggestive of a drug effect.

The objection may also be raised that the observations in the present study were made with an unrecognized categorization of the manifestations of multiple sclerosis. The classification used, however, is based on the evaluation of the disabilities of 300 patients, and the same criteria were used for both the comparison and the isoniazid patients.

Since discharge, the isoniazid group has been followed for twelve to seventeen months (8 patients), six through eleven and five-tenths months (9 patients), four through five and five-tenths months (9 patients), and less than four months (4 patients). There has been no new attack, no regression of status, no new abnormality. Some of the more severely handicapped patients are still improving while continuing to receive isoniazid.

Isoniazid (isonicotinic acid hydrazide) (7) is an agent which is markedly inhibitory for tubercle bacilli. The drug is almost completely absorbed from the digestive tract, and one-half to three-fourths of a single dose is excreted in the urine within twenty-four hours. The peak serum concentration is attained one to three hours after an oral dose. It is generally nontoxic for humans in daily doses up to 5 mg. per kg., and the suggested range of therapy in tuberculosis is

3 to 5 mg. per kg. daily. In man (8*a*), the cerebrospinal fluid concentrations of isoniazid have been found to be high; the drug easily passes the "blood brain barrier." In cerebrospinal fluids with no evidence of infection, concentrations of 0.5 γ per ml. have been observed three to five hours after the administration of 1.5 to 2 mg. per kg. by mouth (8 *b*); this is slightly higher than simultaneously determined plasma concentrations.

The pharmacologic actions of isoniazid in man are few (9). There is no vasodilator action, despite its apparent chemical relationship to nicotinamide. There is no histamine or antihistamine activity and no effective autonomic action.

It is possibly relevant to the present study that isoniazid can affect neural tissue to induce seizures and peripheral neuropathy. Indeed, the effects on the nervous system are the chief side reactions of isoniazid although a miscellany of untoward reactions has been recorded (10). Some of the latter instances are perhaps attributable to the earlier confusion of isoniazid with the more toxic isopropyl derivative of the hydrazide (iproniazid). Pyridoxine seems to counteract the deleterious nervous system effects of isoniazid (11); isoniazid increases the urinary excretion of pyridoxine. The incidence of damage to the peripheral nerves appears to vary inversely with the degree of *in vivo* acetylation of the drug, a process which has a marked but individual variation (12).

In recent investigations (13, 14), it has been reported that there is no effect of isoniazid on mood or mentation. Hence, it seems improbable that improvement in the manifestations of illness of the patients of the present study can be attributable to euphoria.

Thus, although there is no rationale immediately at hand for the use of isoniazid in multiple sclerosis, it can be said that the drug is known to have a definite influence on the nervous system when administered in a sufficient dosage. Moreover, the safety of isoniazid for humans in the doses used in the present study has been well established from the studies in the chemotherapy of tuberculosis. Hence, the continued investigation of isoniazid in multiple sclerosis need not await long-term studies of its toxicity in man.

It is suggested that isoniazid and other related hydrazides be tried in the experimental allergic demyelinating encephalomyelitides. Therapeutic efficacy could easily be demonstrated; and, in such conditions, without entering into the relationship of these conditions to multiple sclerosis, a better drug than isoniazid might more readily be found—if isoniazid should prove to be an active therapeutic agent in multiple sclerosis.

Further work with isoniazid in multiple sclerosis is indicated. The proof of efficacy lies in blind placebo-and-isoniazid regimens prescribed on a random basis in different centers. By this means it will be possible to compare the degree of improvement as well as the per cent improved. It remains to be ascertained whether the drug should be taken continuously, like insulin, or merely for each episode as with penicillin. One must investigate whether there is "escape" from the efficacy of isoniazid, as occurs in certain circumstances with tubercle bacilli, or whether it is an active prophylactic agent and will prevent exacerbations.

The dosage must also be established. Here the individual variation of the degree of acetylation must be correlated with the apparent effect or its failure to appear. Long-term toxicity, although unlikely, must be considered, and there is one point in this connection which is worthy of emphasis. There is reason to believe, from experience in tuberculosis chemotherapy, that seizures as a toxic reaction to isoniazid are more apt to be precipitated in epileptics or in persons with localized lesions in the brain than in other persons. As a consequence, if isoniazid is to be used in patients with neurologic disease, this possibility should be kept in mind and appropriate precautionary measures taken.

These various problems await solution; the goal of an effective treatment for multiple sclerosis may then be closer to attainment.

SUMMARY

Thirty patients with multiple sclerosis were given isoniazid during hospitalization. With one exception, all of the patients had experienced an episode of activity of this disease within a two-year period prior to the use of isoniazid. These 30 patients were compared with 175 patients with multiple sclerosis admitted between 1944 and 1953 and similarly treated except for the administration of the isoniazid.

Of the isoniazid group, 90 per cent of the patients improved, 7 per cent were unchanged, and 3 per cent were worse. Of the comparison group, 33 per cent of the patients improved, 50 per cent were unchanged, and 17 per cent were worse on discharge. The difference between the two groups is significantly well beyond the 0.1 per cent level ($P < 0.001$).

On admission, 33 per cent of the comparison group and 53 per cent of the isoniazid-treated group were unable to walk unaided; on discharge this disability rate was unchanged in the comparison group but, in the group treated with isoniazid, the patients who required assistance in walking had dropped to 33 per cent of the total.

Of the comparison group admitted with mild to moderate disabilities (categories 1 through 5), a total of 116 patients (50 per cent) were unchanged at the time of discharge, 26 per cent were improved by one or more categories, and 24 per cent were worse. In the group treated with isoniazid, 11 were in the mild to moderate categories on admission (categories 1 through 5), but on discharge 100 per cent of these had improved.

In the categories of moderate to severe impairment (categories 6 through 10), there were 58 patients in the comparison group. Of this group, 53 per cent were unchanged, 24 per cent were worse, and 23 per cent had improved during hospitalization. In the group treated with isoniazid, there were 19 patients in categories 6 through 10. Of these, 85 per cent improved, 10 per cent were unchanged, and 5 per cent (one patient) died while in the hospital.

Those with minimal or no symptoms on discharge constituted 27

per cent of the comparison group and 50 per cent of the isoniazid-treated group, although the isoniazid-treated group was more severely disabled than the comparison group at the time of admission.

Four patients showed improvement on isoniazid, worsening with cessation of administration, and then improvement concomitant with reinstitution of the drug. Three other patients had no exacerbation after removal of this agent prior to discharge.

The two groups were comparable at the time of admission in age, age of onset, and duration of illness.

Improvement has been maintained for one to one and one-half years in 8 patients and from one-half to one year in 9 patients of the isoniazid group. The remainder have not been followed as long after discharge from the hospital.

It is concluded that, in the present series of patients, the administration of isoniazid seemed to exert a favorable influence on those manifestations of multiple sclerosis which had been present for less than two years when the isoniazid administration was initiated.

SUMARIO

Los Efectos de la Isoniacida en los Enfermos de Esclerosis en Placas

Treinta enfermos que tenían esclerosis en placas recibieron isoniacida durante su hospitalización. Con una excepción, todos habían experimentado un episodio de actividad de la dolencia durante el período de dos años anterior al uso de la droga. Esos 30 enfermos fueron comparados con 175 enfermos de esclerosis en placas recibidos entre 1944 y 1953 y tratados en forma semejante, excepto por la administración de isoniacida.

Del grupo de la isoniacida, 90 por ciento mejoraron, 7 por ciento no variaron y 3 por ciento empeoraron. Del grupo de comparación, 33 por ciento mejoraron, 50 por ciento no variaron y 17 por ciento empeoraron o fueron dados de alta. La diferencia entre los dos grupos queda significativamente bien más allá del tenor de 0.1 por ciento ($P < 0.001$).

Al ingreso, 33 por ciento del grupo de comparación y 53 por ciento del tratado con isoniacida no podían caminar sin ayuda; al darlos de alta, ese coeficiente de incapacidad no había variado en el grupo de comparación, pero en el grupo tratado con isoniacida, los sujetos que necesitaban ayuda para caminar habían descendido a 33 por ciento del total.

En el grupo de comparación, considerando los recibidos con incapacidad leve a moderada (categorías 1 a 5 inclusive), un total de 116 enfermos (50 por ciento) no habían variado al darlos de alta, 26 por ciento habían mejorado hasta una o más categorías y 24 por ciento habían empeorado. En el grupo de la isoniacida, 11 correspondían a su ingreso a las clases leve a moderada (categorías 1 a 5 inclusive), pero al darlos de alta, 100 por ciento de ellos habían mejorado.

En las categorías de incapacidad moderada a grave (categorías 6 a 10 inclusive), hubo 58 enfermos del grupo de comparación. De ellos, 53 por ciento no variaron, 24 por ciento empeoraron y 23 por ciento mejoraron durante la hospitalización. Del grupo tratado con isoniacida, 19 enfermos quedaban en las categorías 6 a 10 inclusive. De ellos, 85 por ciento mejoraron, 10 por ciento no variaron y 5 por ciento (un enfermo) fallecieron en el hospital. Los que tenían síntomas mínimos o no tenían síntomas al darlos de alta constituían 27 por ciento del grupo de comparación y 50 por ciento del tratado con isoniacida, aunque al ingreso, los últimos estaban más incapacitados que los del otro grupo.

Cuatro enfermos revelaron mejoría con la isoniacida, empeoramiento al cesar la administración y mejoría de nuevo al reanudarse la droga. Otros tres enfermos no manifestaron exacerbación al suspenderse dicho agente antes del alta.

Los dos grupos eran comparables en la fecha del ingreso en cuanto a edad, edad a la iniciación y duración de la enfermedad.

Se ha mantenido la mejoría de un año a año y medio en 8 enfermos y de medio a un año en 9 enfermos del grupo de la isoniacida. Los demás no han sido mantenidos en observación tanto tiempo después del alta del hospital.

Dedúcese que, en la serie actual de enfermos, la administración de isoniacida pareció ejercer un influjo favorable sobre las manifestaciones de la esclerosis en placas que habían existido menos de dos años al iniciarse la administración de isoniacida.

RESUME

Les effets de l'isoniazide sur les malades atteints de sclérose en plaques

Trente patients atteints de sclérose en plaques ont été traités par l'isoniazide au cours de leur séjour à l'hôpital. A l'exception d'un seul cas, tous les malades avaient présenté une période d'activité de l'affection au cours des deux années précédant l'emploi de l'isoniazide. Une étude comparative a été effectuée entre ces 30 cas et 175 autres cas de sclérose en plaques hospitalisés entre 1944 et 1953 et qui avaient été traités de façon similaire, sauf l'administration de l'isoniazide.

Parmi le groupe traité par l'isoniazide, 90% des cas ont été améliorés, 7% n'ont présenté aucun changement, 3% étaient aggravés au sortir de l'hôpital. Parmi le groupe témoin, 33% des cas étaient améliorés, 50% sans changement, 17% étaient aggravés au sortir de l'hôpital. L'écart entre les deux groupes dépasse largement la proportion significative de 0,1 pour 100 ($P < 0{,}001$).

À l'admission, 33% du groupe de comparaison et 53% du groupe traité par l'isoniazide ne pouvaient marcher sans aide. Au sortir de l'hôpital, cette proportion persistait pour le groupe de comparaison et elle s'abaissait pour le groupe traité par l'isoniazide, 33% des malades seulement avaient encore besoin d'assistance pour marcher.

Parmi le groupe de comparaison présentant à l'admission une invalidité légère ou modérée (catégories 1 à 5 inclusivement), 116 cas en tout, soit 50%, ne présentaient aucun changement à la sortie de l'hôpital, 26% étaient améliorés d'un ou plusieurs degrés et 24% étaient aggravés. Dans le groupe traité par l'isoniazide, 11 figuraient dans les catégories d'invalidité légère ou modérée (catégories 1 à 5 inclusivement) à l'admission, mais à la sortie de l'hôpital 100% de ces cas étaient améliorés.

Dans les catégories d'invalidité modérée ou sévère (catégories 6 à 10 inclusivement) il y avait 58 patients dans le groupe de comparaison. Parmi ce groupe, 53% des cas étaient sans changement, 24% étaient aggravés, et 23% s'étaient améliorés au cours de l'hospitalisation. Dans le groupe traité par l'isoniazide, il y avait 19 patients appartenant aux catégories 6 à 10 inclusivement. Parmi ceux-ci, 85% des cas étaient améliorés, 10% étaient sans changement, et 5% (1 malade) représentent les décès au cours de l'hospitalisation.

Les cas dont les symptômes étaient minimes ou nuls à la sortie de l'hôpital représentaient 27% du groupe de comparaison et 50% du groupe traité par l'isoniazide, bien qu'à l'admission l'invalidité ait été beaucoup plus accusée parmi le groupe traité par l'isoniazide que dans le groupe témoin.

Quatre patients ont présenté une amélioration avec l'isoniazide, une aggravation lorsque l'administration du composé a été interrompue, puis une amélioration associée à la reprise de cette médication. Trois autres patients n'ont présenté aucune exacerbation de l'affection lorsque la médication a été interrompue avant la sortie de l'hôpital.

Les deux groupes étaient comparables au moment de l'admission en ce concerne l'âge du malade, l'âge auquel la maladie avait débuté et la durée de l'affection.

L'amélioration a été maintenue pendant un an à un an et demi, chez 8 patients, et pendant six mois à un an, chez 9 patients du groupe traité par l'isoniazide. Les autres n'ont pas été suivis pendant une période aussi longue après la sortie de l'hôpital.

En conclusion, dans la série actuelle de malades, l'administration de l'isoniazide a paru exercer une influence favorable sur les symptômes de la sclérose en plaques qui existaient depuis moins de deux années au moment de l'instauration du traitement par l'isoniazide.

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UNDETECTED TUBERCULOSIS IN VARIOUS ECONOMIC GROUPS[1]

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INTRODUCTION

A number of studies have demonstrated an inverse relationship between tuberculosis and economic status. Using occupation as a measure of economic status, Britten (1) analyzed tuberculosis mortality rates in 1930 among males in 10 states and showed the tuberculosis mortality rate to be approximately seven times as great among unskilled workers as among professional men. Terris (2), using a combination of indices to determine relative economic status, established that the death rate among males was three times as high in his lowest "economic quarter" as in his highest; and among females, twice as high. Other studies, utilizing mortality data, have shown similar results (3, 4, 5). The National Health Survey, conducted in the United States in 1935–1936, concluded that tuberculosis known to the individual was about four times as prevalent in the lowest income group as in the highest (6).

Reports such as these have influenced tuberculosis case-finding efforts in this country and have focused attention on the lower economic groups. Such studies may be misleading when the results are used to direct case-finding programs, for the productivity of case finding varies directly with the prevalence of undetected tuberculosis or early cases rather than with the incidence of death or prevalence of known disease. Because tuberculosis is likely to cause considerable economic distress during its course, the economic status of patients is likely to change as the disease progresses. End point measures, such as mortality, should show a somewhat different economic distribution of disease, therefore, than measures of the disease earlier in its course.

PLAN OF STUDY

To test the hypothesis suggested above, i.e., that undetected cases are more prevalent in the higher economic levels than deaths or known cases would suggest, data available from a community-wide chest roentgenographic survey conducted in Cuyahoga County (including the city of Cleveland), Ohio, in 1949, from the health department records and from the 1950 census, were studied. In the Cuyahoga County chest roentgenographic program, 59 per cent of the eligible resident population (fifteen years of age and older) received roentgenographic examinations during a twenty-four-week period (7). The roentgenograms were interpreted by physicians trained in radiology and, if considered abnormal, the person concerned was referred for diagnostic study. Of a total of 634,877 satisfactory examinations on residents of the County, 7,695 were considered positive for tuberculosis on the basis of their initial films and 4,033 were subsequently

[1] From the Division of Chronic Disease and Tuberculosis, Public Health Service, U. S. Department of Health, Education, and Welfare, Washington, D. C.

TABLE 1

NUMBER OF CENSUS TRACTS INCLUDED IN EACH ECONOMIC FIFTH AND THEIR 1950 POPULATIONS. CUYAHOGA COUNTY, OHIO

Economic Fifth	Number of Census Tracts	Total Population	Population Fifteen Years of Age and Older
Lowest	78	292,833	225,204
Second	63	277,256	211,308
Third	61	274,336	213,801
Fourth	71	269,077	202,012
Highest	68	276,030	212,037

TABLE 2

RESPONSE TO ROENTGENOGRAPHIC SURVEY PROGRAM IN CUYAHOGA COUNTY, OHIO, IN 1949, BY ECONOMIC GROUP

Economic Fifth	Population Fifteen Years of Age and Older	Examined on Survey Fifteen Years of Age and Older	Per Cent Examined on Survey
Lowest	225,204	143,861	63.9
Second	211,308	127,513	60.3
Third	213,801	127,350	59.6
Fourth	202,012	117,174	58.0
Highest	212,037	116,351	54.9

diagnosed by physicians and clinics as having tuberculosis. Of this group of tuberculous persons, 3,398 (84 per cent) were previously unknown to the local health department. These 3,398 persons represent undetected cases of tuberculosis which chest roentgenographic programs are primarily designed to uncover.

All records from the survey were tabulated by age, race, sex, and census tract[2] of residence. No attempt was made to ascertain the economic status of the examinees on the survey records. The 1950 census, however, provides a basis for estimating the economic status of the groups responding. Material prepared by Green (8) was used for this purpose. He had arrayed all census tracts in Cuyahoga County by median family income as obtained in the 1950 census and thus identified each census tract with an economic tenth. To facilitate analysis of data presented here, these tenths were regrouped into fifths, with the results shown on table 1. Approximately one-fifth of the families in Cuyahoga County live in the census tracts included in each economic fifth. With census tracts thus identified, records from the roentgenographic survey were also grouped into economic fifths on the basis of the census tract in which the examinee lived.

The response to the roentgenographic program varied with economic level, as may be seen in table 2, ranging from a 63.9 per cent response among persons in the lowest level to 54.9 in the highest. Although this range is not great, it may

[2] Census tracts are fairly homogeneous areas, having a population usually between 3,000 and 6,000, into which certain large cities (and sometimes their adjacent areas) have been subdivided for statistical and local administrative purposes.

reflect less interest by the higher economic groups in participating in the survey —or perhaps the roentgenographic program was less strongly directed toward these groups.

OBSERVATIONS

The prevalence of tuberculosis suspected on the basis of the screening roentgenogram, the prevalence of diagnosed tuberculosis, and the prevalence of previously undetected diagnosed tuberculosis in each economic level are shown in table 3. The diagnostic studies appeared to be equally complete in all economic levels, with approximately 50 per cent of the tuberculosis suspects in each economic level confirmed as cases of tuberculosis. The prevalence rates shown for diagnosed tuberculosis probably truly reflect, therefore, the relative prevalence of tuberculosis at the time the survey was conducted in the population examined from each economic level.

The prevalence of previously undetected tuberculosis among the population responding for examination varied significantly with economic level both in total and for the "active" group. Tuberculosis with activity undetermined has been included with the cases designated *active* and *probably active* because, on the basis of a previous study (9), it is believed that these two groups of cases would ultimately prove to be of similar public health significance. For the "active" group, more than twice as much disease existed in the lowest economic group as in the highest. The large difference between the lowest and second economic fifth is of interest and could be due to a heavy weighting of nonwhites in the population examined from the lowest economic fifth. In the lowest economic fifth, the prevalence of "active" tuberculosis was nearly twice as high among nonwhites as whites. In cities with a smaller portion of nonwhite population, possibly a smaller differential between economic fifths would be observed.

The distribution of tuberculosis deaths in Cuyahoga County during the year 1950 and the distribution of cases of tuberculosis known to the health department as of December 31, 1950, are shown in table 4 and figure 1. Shown in the last

TABLE 3

PREVALENCE OF TUBERCULOSIS SUSPECTED AND DIAGNOSED IN POPULATIONS EXAMINED FROM EACH ECONOMIC GROUP

Economic Fifth	Rates per 1,000 Examinations				
	Roentgenographic Film Suspicious of Tuberculosis (1)	Diagnosed Tuberculosis (2)	Diagnosed Tuberculosis Previously Undetected		
			Total (3)	Active* (4)	Inactive (5)
Lowest	15.7	7.8	6.4	2.5	3.9
Second	12.4	6.5	5.3	1.5	3.8
Third	12.7	6.3	5.3	1.4	3.9
Fourth	10.6	5.6	4.8	1.2	3.6
Highest	9.5	5.3	4.6	1.1	3.5

* Includes probably active and activity undetermined.

TABLE 4

DISTRIBUTION OF TUBERCULOSIS DEATHS, KNOWN CASES, AND ESTIMATED UNDETECTED CASES IN CUYAHOGA COUNTY, OHIO

Economic Fifth	Tuberculosis Deaths 1950		Tuberculosis Cases Known to Health Department as of December 31, 1950		Undetected Tuberculosis (Estimated from Survey) Per Cent
	Number	Per Cent	Number	Per Cent	
All Levels	300	100.0	12,655	100.0	100.0
Lowest	177	59.0	4,435	35.0	25.6
Second	51	17.0	2,635	20.8	19.8
Third	39	13.0	2,288	18.1	20.1
Fourth	24	8.0	1,791	14.2	17.2
Highest	9	3.0	1,506	11.9	17.3

column of the table is the estimated percentage distribution of cases of undetected tuberculosis which existed in Cuyahoga County at the time the roentgenographic survey was conducted, derived by applying the prevalence rates found on the survey to the population fifteen years of age and older in each economic fifth. Thirty per cent of the survey-discovered cases were "active" while, of the cases known to the health department, approximately a third were "active." Thus, the differences in the distributions of known cases and undetected cases shown in table 4 are probably not due to differences in the kinds of tuberculosis included in each group.

The prevalence rates used in deriving the estimated distribution of undetected tuberculosis shown in table 4 and figure 1 represent what was actually found in a population that responded to a chest roentgenographic survey. Hence, it is believed that, at least for directing chest roentgenographic programs, this distribution of undetected tuberculosis is a realistic estimate of the importance of the various economic levels as a source for undetected tuberculosis.

The application of these data can be greatly enlarged if it can be assumed either (*a*) that the prevalence of undetected tuberculosis was the same in the total population of each economic level as in the population examined, or (*b*) that the estimated prevalence of undetected tuberculosis in the total population is over- or understated to the same extent in each economic level. There is no way of completely testing either of these assumptions. It is probable that the prevalence of undetected tuberculosis in the surveyed population is actually an underestimate of the prevalence in the total population since response was poor from the older age groups, in which a high prevalence of disease was found (7). The population examined from each economic level was about equally representative of the population available in terms of age, sex, and race, however, so that there is some assurance that, even if the prevalence rates in the surveyed population understate the prevalence rates in the total population, the bias is present to the same extent in each of the five economic groups. It seems quite likely that the difference between the distribution of tuberculosis as shown by the roentgenographic survey and the distribution shown by other types of measure is due to

the type of measure applied rather than to differences in the response of the population from each economic level to the roentgenographic program.

Mortality data are obviously a poor measure of the relative prevalence of undetected tuberculosis in various economic levels. The data available suggest that there is approximately twenty times as much tuberculosis in the lowest economic level as in the highest, as compared with only one and a half times as much in the lowest economic group, as estimated from the chest roentgenographic survey. Data on tuberculosis known to the health department appears to be a considerably better measure, although it also tends to de-emphasize the higher economic groups as a source for undetected tuberculosis. In connection with the data on cases of tuberculosis known to the health department on December 31, 1950, it should be pointed out that these were obtained after the survey had been conducted, with the result that they are weighted by the survey findings. Unfortunately, data regarding registered tuberculosis cases by economic level just prior to the roentgenographic survey were not available for the present report. It is likely, however, that such data would tend to emphasize the lower economic levels considerably more than those shown here. If, for example, cases discovered by the survey were subtracted from the cases known to the health department at the end of 1950, an even larger proportion of the known cases would fall in the lower economic groups.

The estimated distribution of undetected *active* cases is not shown in table 4 because data regarding "active" cases known to the health department were not readily available for comparison by economic level. The discovery of these cases is a primary objective in roentgenographic case-finding programs. The estimated distribution of undetected "active" cases of tuberculosis (obtained by

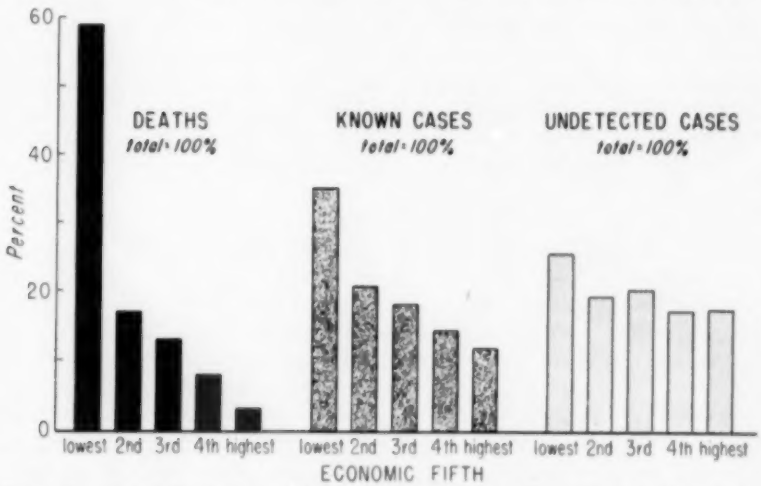

FIG. 1. Percentage distribution of deaths from tuberculosis, known cases, and undetected cases, by economic fifth, Cuyahoga County, Ohio.

multiplying the population fifteen years of age and older shown in table 1 by the corresponding prevalence rates shown in column 4 of table 3) is as follows: in the lowest economic fifth, 5,630 cases (34.0 per cent of the total); second economic fifth, 3,170 cases (19.2 per cent); third economic fifth, 2,993 (18.1 per cent); fourth economic fifth, 2,424 cases (14.6 per cent); and in the highest economic fifth, 2,332 cases (14.1 per cent).

It can be concluded, therefore, that undetected cases of tuberculosis are more prevalent in the higher economic levels than either deaths or known cases would suggest. This may be due to a changing economic status of patients with tuberculosis as the disease progresses, as originally suggested, or it may be due to other reasons. Possibly tuberculosis is more chronic in the higher economic groups, less frequently terminating in death and less frequently appearing to be of sufficient clinical significance to warrant a notification to the health department. Regardless of the reason, the higher economic groups should not be overlooked in the conduct of tuberculosis control programs and, in particular, in chest roentgenographic surveys.

While roentgenographic detection programs in lower economic levels are no doubt more productive in terms of the amount of disease discovered than programs in the higher levels, a concentration of case-finding efforts in the lower levels would leave an appreciable amount of disease undetected. If the chest roentgenographic survey in Cuyahoga County had concentrated on only the lowest economic fifth of the population and had succeeded in examining that group completely, it is probable that only one-third of the "active" tuberculosis in the county would have been brought to light while, of the total undetected cases of tuberculosis in Cuyahoga County (including inactive), a complete roentgenographic examination of this lowest economic fifth could have produced only a quarter. Moreover, case for case, those in the higher economic groups may be the more important, if they are indeed the more chronically ill, for they possibly represent important residual sources of infection. In this light, the mass approach for tuberculosis case finding continues to be a productive and necessary part of our control effort. Tuberculosis can be found in all economic groups. No one can consider himself economically immune to tuberculosis.

SUMMARY

On the basis of data regarding deaths from tuberculosis and the prevalence of known cases of tuberculosis, there appears to be a pronounced relationship between the disease and measures of economic status.

Because tuberculosis causes considerable economic distress during its course, it is possible that undetected or early tuberculosis may be distributed somewhat differently among economic groups than are deaths or known cases.

Data from a community-wide chest roentgenographic program in Cleveland, Ohio, in which approximately 60 per cent of the eligible population were examined, are used to estimate the distribution of undetected tuberculosis among various economic groups.

While the prevalence of previously undetected tuberculosis varied significantly with the economic level, it was much more uniformly distributed among economic levels than were either deaths from tuberculosis or known cases of tuberculosis. Whereas deaths suggested approximately twenty times as much tuberculosis in the lowest economic level as in the highest, and known cases suggested three times as much, the estimated distribution of undetected cases showed only one and a half times as much.

It is concluded that the higher economic groups should not be overlooked in tuberculosis detection programs and that the mass approach to tuberculosis case finding is a productive and necessary part of our control effort.

SUMARIO

La Tuberculosis Oculta en Varios Grupos Económicos

A base de los datos relativos a las muertes por tuberculosis y y la frecuencia de los casos conocidos de tuberculosis, parece que existe una notable relación entre la enfermedad y los índices del estado económico.

Por ocasionar la tuberculosis considerable perturbación económica durante su evolución, hay buenos motivos para creer que la tuberculosis oculta o incipiente quizás muestre una distribución algo distinta de la de las muertes o los casos conocidos entre los varios grupos económicos.

Los datos arrojados por una encuesta radiográfica colectiva en la ciudad de Cléveland, Ohio, en la que se examinó el tórax aproximadamente a 60 por ciento de la población eligible, han sido tomados para calcular la distribución de la tuberculosis oculta en los varios grupos económicos.

Aunque la frecuencia de la tuberculosis no descubierta antes varió notablemente de acuerdo con el nivel económico, estaba distribuída mucho más uniformemente entre los distintos grupos que ya las muertes o los casos conocidos de tuberculosis. En tanto que las muertes sugerían unas veinte veces más tuberculosis al nivel económico más bajo comparado con el más alto, y los casos conocidos tres veces más, la distribución calculada de los casos ocultos reveló solamente una y media veces más.

Dedúcese que no debe pasarse por alto a los grupos económicos más elevados en las obras de descubrimiento de la tuberculosis y que el ataque colectivo en la busca de casos debe constituir parte productiva y necesaria de nuestros planes de lucha.

RESUME

Tuberculose non dépistée dans divers groupes économiques

Sur la base de données relatives aux décès par tuberculose et la prédominance des cas reconnus, il paraît exister un rapport marqué entre la maladie et la valeur des conditions économiques.

Etant donné que la tuberculose crée des conditions économiques extrêmement défavorables au cours de son évolution, il y a tout lieu de croire que la répartition de la tuberculose inapparente ou des cas non dépistés parmi les divers groupes économiques s'effectue d'une manière assez différente de celle des décès ou des cas dépistés.

Les données fournies par le programme de dépistage radiologique, effectué à l'échelle municipale par la ville de Cleveland, Ohio, au cours duquel environ 60% de la population éligible furent examinés, sont utilisées pour évaluer la distribution des cas de tuberculose non dépistés parmi les divers groupes économiques.

Tandis que la prédominance de la tuberculose antérieurement non décelée variait de

façon significative selon le niveau économique, elle était répartie dans les divers groupes économiques avec beaucoup plus d'uniformité que les décès par tuberculose ou les cas de tuberculose reconnus. Bien que le nombre des décès implique approximativement vingt fois plus de cas de tuberculose dans le groupe économiquement le plus faible, par comparaison avec le plus élevé, et que le nombre des cas reconnus en suggère trois fois plus, les computations relatives à la distribution des cas non dépistés n'en montraient qu'une fois et demie autant.

La conclusion de ces données est qu'il ne faut pas négliger d'inclure les groupes économiquement élevés dans les programmes de dépistage et que ceux-ci doivent être abordés sous l'aspect collectif, qui représente une phase féconde et nécessaire du contrôle de la tuberculose.

Acknowledgment

The writers are grateful to Dr. Joseph Stocklen, Tuberculosis Controller for Cuyahoga County, Ohio, for permission to use data from his files.

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PULMONARY TUBERCULOSIS RELAPSE AND MORTALITY[1]

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INTRODUCTION

This is a report of the after-history of 1,213 tuberculous patients who began their first treatment for pulmonary tuberculosis between 1919 and 1944 at Mt. McGregor Sanatorium in Upstate New York and were followed through 1949. The study is divided in three parts: the status (alive or dead) at completion of first treatment; the number of relapses of tuberculosis after completion of the first treatment; and the mortality after completion of the first treatment.

The Metropolitan Life Insurance Company maintained a sanatorium at Mt. McGregor, New York, from November, 1913, to September, 1945, where its employees who became ill with active or questionably active pulmonary tuberculosis were invited for treatment. They came from the Field force, or selling force, in the United States and Canada east of the Rocky Mountains, and from the clerical staffs at the Home Office in New York City and the Head Office in Ottawa, Canada.

They received their treatment under optimal conditions: during residence they were under no expense for their treatment, most of them were eligible for and received disability insurance benefits throughout their illness, and they were assured of re-employment with the Company when they completed treatment.

Before their illness they had been under medical supervision while in the service of the Company. Every employee had an annual physical checkup. Those in the Field were examined by doctors in their communities, and the reports were forwarded to the Home Office or Head Office for review and recommendations. At the Home Office, fluoroscopic examination of the thorax was added to the annual physical examination in 1928 and a roentgenogram was made when indicated. In many instances, tuberculosis was detected before signs or symptoms appeared. Consequently, since 1928 the proportion of cases detected in the minimal stage at the Home Office has increased and most of them were diagnosed early in their disease.

The Sanatorium followed these people through 1949. After discharge from Mt. McGregor, a letter was sent annually to each expatient requesting information about his health during the preceding year. After varying periods of follow-up, a few expatients failed to reply. The lack of information is pointed out when it might affect the results of the study. On the whole, the follow-up has been successful and invaluable.

COMPOSITION OF THE GROUP

Patients resided at Mt. McGregor for at least three months or completed treatment within that time. The period of three months was chosen arbitrarily,

[1] From the Medical Division, Metropolitan Life Insurance Company, New York, New York.

for it was thought that those who left the Sanatorium against advice after a shorter period did not represent patients who had had sufficient instruction or supervision to take care of themselves properly.[2]

The group consists of 648 males and 565 females (*see* table 1). The median age of the males fell in the group thirty to thirty-four years; of the females, twenty to twenty-four years. This difference in age distribution is due largely to the difference in age distribution of the total employee group.

Among the males, 27.1 per cent began treatment when the pulmonary tuberculosis was in the minimal stage, 49.6 percent when it was in the moderately advanced stage, and 23.3 percent when the disease was in the far advanced stage.[3] Among the females, the percentages were 45.0 per cent minimal, 42.3 per cent moderately advanced, and 12.7 per cent far advanced tuberculosis. The higher percentage of minimal cases among the females is due partly to the fact that the majority of the females came from the Home Office, where the proportion of cases detected in the minimal stage was higher.

TERMINATION OF FIRST TREATMENT

The percentage of patients whose first treatment was terminated by death increased with the increase in the stage of tuberculosis on admission (table 1) for both the males and the females.

Very few with minimal tuberculosis died (males, 1.1 per cent; females, 3.1 per cent). Considerably more with moderately advanced tuberculosis died (males, 16.2 per cent; females, 20.1 per cent). Among those with far advanced tuberculosis on admission to the hospital, 44.4 per cent of the males and 56.9 per cent of the females died. Although the rates for females appear higher, the difference is not statistically significant but may be due to chance. Among the males with far advanced tuberculosis, the percentage of deaths was higher in the age group of twenty-five years or older on admission than in the age group of less than twenty-five years; among those in the moderately advanced stage, there was some indication that the percentage of deaths increased with age on admission. Age on admission did not seem to be a factor in the deaths among females.

The percentage of deaths in each stage decreased in the later years of admission. The percentage of deaths at each stage for admissions in 1919–1927, 1928–1933, 1934–1944 are shown in table 2.

It will be noted also that in each period the percentage of deaths among those admitted in the minimal stage was considerably lower than among those admitted in the advanced stages of disease.

[2] In a previous report "Follow-up of 1,041 Tuberculous Patients" by the writer (Am. Rev. Tubere., 1941, 44, 451), the patients selected were at Mt. McGregor for at least four months. This report includes that group of 1,041 plus 15 expatients who resided at Mt. McGregor for at least three months but less than four months, 36 clerks from the Canadian Head Office, and 121 admissions in 1939 through 1944.

[3] The stage of disease (minimal, moderately advanced, and far advanced) is defined in the *Diagnostic Standards and Classification of Tuberculosis* of the National Tuberculosis Association.

TABLE 1

SEX, AGE, AND STAGE OF DISEASE AT BEGINNING OF FIRST HOSPITAL TREATMENT FOR PULMONARY TUBERCULOSIS

Number and Percentage Who Terminated Treatment by Death

Sex and Age on Admission	Minimal			Moderately Advanced			Far Advanced		
	Patients	Deaths	Per Cent	Patients	Deaths	Per Cent	Patients	Deaths	Per Cent
Males	176	2	1.1	321	52	16.2	151	67	44.4
17–24	17	—	—	42	6	14.3	43	11	25.6
25–34	72	—	—	107	21	19.6	62	30	48.4
35–49	68	—	—	135	15	11.1	42	24	57.1
50–64	19	2	10.5	37	10	27.0	4	2	50.0
Females	254	8	3.1	239	48	20.1	72	41	56.9
17–19	25	—	—	33	9	27.3	20	13	65.0
20–24	125	5	4.0	137	24	17.5	34	20	58.8
25–29	60	3	5.0	45	10	22.2	7	3	42.7
30–59	44	—	—	24	5	20.8	11	5	45.5

The marked difference in the experience of the minimal cases and advanced cases is dramatic indication of the value of detecting tuberculosis and starting hospital treatment in an early stage.

RELAPSE OF TUBERCULOSIS

After patients had completed their treatment at Mt. McGregor, the Sanatorium doctor recommended return to work. If they continued rest treatment at home, their own doctors recommended return to work on completion of the period of rest treatment. Approximately 75 per cent completed their rest treatment at Mt. McGregor, and the others for various reasons completed their treatment under the care of their own physicians. After their return to work the majority did well, but some had relapses of their tuberculosis and had to undergo rest treatment again.

In this study, "relapse" means exacerbation of tuberculosis with discontinuance of work and resumption of rest treatment.[4] Rates of relapse should not be confused or compared with readmission rates. Those who had relapses were treated at Mt. McGregor, other sanatoriums, or at home. The relapse rates are necessarily higher than readmission rates. It should be noted also that the follow-up observations began when the rest treatment was completed and the patient was able to return to work.

Relapse rates are shown in table 3 for each of the first ten years after completion of the first hospital treatment. They are based on the number of persons followed each year after completion of treatment regardless of the calendar year. For example, 174 males admitted to Mt. McGregor with tuberculosis in

[4] In the previous report, the term "recurrence" was used. It is thought that "relapse" is more accurate.

TABLE 2

PATIENTS WHO TERMINATED FIRST TREATMENT BY DEATH, BY SEX, STAGE OF DISEASE, AND YEAR OF FIRST ADMISSION

Sex and Year of Admission	Minimal			Moderately Advanced			Far Advanced		
	Patients	Deaths	Per Cent	Patients	Deaths	Per Cent	Patients	Deaths	Per Cent
Males	176	2	1.1	321	52	16.2	151	67	44.4
1919–1927	75	2	2.7	149	34	22.8	74	38	51.4
1928–1932	42	—	—	80	9	11.3	42	17	40.5
1933–1944	59	—	—	92	9	9.8	35	12	34.3
Females	254	8	3.1	239	48	20.1	72	41	56.9
1919–1927	54	3	5.6	85	31	36.5	42	24	57.1
1927–1932	92	1	1.1	77	9	11.7	21	13	61.9
1933–1944	108	4	3.7	77	8	10.4	9	4	44.4

the minimal stage were followed for at least one year after completion of hospital treatment; 17 (9.8 per cent) had a relapse of their tuberculosis in that year. In the second year after completion of treatment, 156 males were followed and were liable to a first relapse of their tuberculosis. Those who had a relapse in the first year were not counted in the follow-up for the second year since they had had their first relapse; an additional man had completed his treatment in 1948 and therefore had only one year of follow-up. In the second year, 13 of 156 (8.3 per cent) males experienced a relapse; and so on for each year of follow-up.

In general, the rate of relapse decreased with the number of years elapsed after completion of hospital treatment. After the tenth year a first relapse was very infrequent but could not be entirely excluded from consideration. One man who was treated in the minimal stage had the first relapse twenty-two years after completion of treatment, and one woman in the moderately advanced stage had a relapse sixteen years after completion of treatment.

Approximately 95 per cent of the patients terminated their rest treatment five years or more before the close of the study. We therefore have a group of 968 whose follow-up observations were complete for a five-year period. Of those who began their treatment in the minimal stage, 28.2 per cent of the males and 15.8 per cent of the females had at least one relapse within five years. The rates were higher in the moderately advanced stage, 37.0 per cent for males and 23.0 per cent for females; and, in the far advanced stage, the relapse rates were 41.6 per cent for males and 19.4 per cent for females.

Age at the beginning of first treatment did not seem to have a very definite influence on the outcome. The only statistically significant difference was among the females in the minimal stage: those who began their hospital treatment when they were less than twenty-five years old had a higher relapse rate of their disease than those who began at twenty-five years or older.

TABLE 3

FIRST RELAPSE OF TUBERCULOSIS AFTER COMPLETION OF TREATMENT BY SEX AND STAGE OF DISEASE AT BEGINNING OF TREATMENT

Year of Follow-up	Minimal			Moderately Advanced			Far Advanced		
	Persons	Relapses	Per Cent	Persons	Relapses	Per Cent	Persons	Relapses	Per Cent
Males									
1	174	17	9.8	269	29	10.8	82	15	18.3
2	156	13	8.3	235	22	9.4	67	6	9.0
3	143	9	6.3	209	21	10.0	61	6	9.8
4	132	5	3.8	186	17	9.1	54	4	7.4
5	127	4	3.1	168	6	3.6	49	3	6.1
6	121	1	.8	154	6	3.9	43	3	7.0
7	110	2	1.8	139	1	.7	39	—	—
8	95	1	1.1	128	2	1.6	38	—	—
9	92	1	1.1	120	2	1.7	35	1	2.9
10	85	—	—	111	2	1.8	31	—	—
Females									
1	245	7	2.9	189	14	7.4	31	1	3.2
2	236	14	5.9	171	10	5.8	30	3	10.0
3	220	5	2.3	161	11	6.8	27	1	3.7
4	211	5	2.4	147	2	1.4	26	—	—
5	204	7	3.4	142	5	3.5	26	1	3.8
6	195	5	2.6	132	2	1.5	23	1	4.3
7	179	3	1.7	121	2	1.7	21	—	—
8	166	2	1.2	111	1	.9	20	—	—
9	155	1	.6	105	1	1.0	20	1	5.0
10	145	1	.7	100	—	—	18	—	—

Length of rest treatment may have had some influence on the subsequent experience. Those who were treated for longer periods of time tended to have lower rates of relapse. On an average, the period of treatment was longer for the females than for the males. This may partly explain the lower rates of relapse for females.

Throughout the years, changes occurred which may have had some effect on the outcome of treatment. After 1928, when annual fluoroscopy was introduced at the Home Office, a larger proportion of the patients began their hospital treatment while the disease was still in the minimal stage and early in their disease. In 1933, at Mt. McGregor, any sputum which was negative for tubercle bacilli on microscopy was inoculated into guinea pigs and, in 1936, gastric washings were similarly inoculated. If the report was unfavorable, the patient was kept at the Sanatorium for a longer time. Since 1932, an increasing number of patients were treated by pneumothorax. The average length of treatment gradually increased over the years.

Persons who began their hospital treatment in the earlier years of this study had a higher relapse rate than those who began in the later years. The hospital admissions were divided into three groups: 1919–1927, 1928–1932, and 1933–

1944. Among males in the minimal stage who began their treatment in the period 1919 to 1927, 36.1 per cent had a relapse of their tuberculosis within five years after completion of treatment, compared with 22.8 per cent who began their treatment in the period 1933–1944. Among the females in the minimal stage, the relapse rate dropped from 21.8 to 9.5 per cent. The rate for males in the moderately advanced stage decreased from 50.9 per cent in the early period to 26.6 per cent, and, in the far advanced stage, 58.8 to 11.1 per cent. The rates for females in the moderately advanced stage dropped from 40.0 to 13.3 per cent, and, in the far advanced stage, from 27.8 per cent to none. The group of females with far advanced disease, however, was too small to yield significant conclusions. Any or all of the changes in detection of cases and the changes in treatment may have affected the relapse rate. Whatever the explanation is, the results of treatment in more recent years show great improvement.

"Lost cases" probably do not affect these results. Only 4 persons were lost in the follow-up in the first five years after completion of cure.

A complete ten-year follow-up is available on 787 persons. Of 815 who completed their treatment in 1939 or prior, 17 (2.1 per cent) have been lost after varying periods of tracing, and an additional 11 (1.3 per cent) were known to be living, but their state of health was not known for part of the ten-year period. The percentage of "lost" or "partially lost" cases is so small that any influence they might have had on the results is not significant (table 4).

The patients who had minimal tuberculosis had a more favorable experience than those with advanced tuberculosis; the difference was especially marked among the males. In each stage, the females had a more favorable experience than the males.

Among the expatients who were followed at least ten years, 209 had one or

TABLE 4

STATUS DURING FIRST TEN YEARS AFTER COMPLETION OF TREATMENT

Status During Ten year Period of Follow-up	Males						Females					
	Minimal		Moderately Advanced		Far Advanced		Minimal		Moderately Advanced		Far Advanced	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	141	100.0	214	100.0	66	100.0	191	100.0	148	100.0	27	100.0
Living	*128*	*90.8*	*184*	*86.0*	*54*	*81.8*	*183*	*95.8*	*126*	*85.1*	*21*	*77.8*
No relapse	86	61.0	108	50.5	31	46.9	144	75.4	100	67.6	18	66.7
1 relapse	28	19.9	53	24.8	20	30.3	32	16.7	19	12.8	2	7.4
2 or 3 relapses	14	9.9	23	10.7	3	4.6	7	3.7	7	4.7	1	3.7
Dead	*13*	*9.2*	*30*	*14.0*	*12*	*18.2*	*8*	*4.2*	*22*	*14.9*	*6*	*22.2*
Cause—Tuberculosis	4	2.8	17	7.9	9	13.6	4	2.1	17	11.5	4	14.8
Cause—Non-tuberculous disease	9	6.4	13	6.1	3	4.6	4	2.1	5	3.4	2	7.4

TABLE 5

MORTALITY OF TUBERCULOUS EXPATIENTS, 1919–1949, COMPARED WITH METROPOLITAN LIFE INSURANCE COMPANY GROUP MORTALITY

Sex and Stage	Number of Expatients	Years of Life Exposed to Risk at Attained Ages 20–69	Actual Deaths			Expected Deaths by M.L.I. Co. Group 1939–1941	Ratio Actual to Expected Deaths
			Total	Tuberculosis	Nontuberculous Disease		
Males							
Minimal	174	2,715	33	12	21	20.155	164
Moderately advanced	269	3,816	64	30	34	26.875	238
Far advanced	82	1,026	23	18	5	5.804	396
Females							
Minimal	246	3,545	13	6	7	5 854	222
Moderately advanced	189	2,401	28	21	7	3.474	806
Far advanced	31	396	7	5	2	0.612	1,144

more relapses and were still living at the end of the ten-year period. One-half of them spent less than two of the ten years under rest treatment again, one-quarter spent two to five years, and one-quarter more than five years.

MORTALITY OF EXPATIENTS

In order to assess the mortality of the expatients, it was necessary to know the mortality of a nontuberculous group which was comparable in age, sex, and occupation. For this comparison, use has been made of the mortality experience of the Metropolitan Life Insurance Company employees insured under the Group Annuity Contract.

Mortality ratios were obtained by calculating the number of deaths expected by the Group Contract rate for quinquennial age groups and dividing them into the number of actual deaths. Among the males who received hospital treatment for tuberculosis in the minimal stage, there were 33 deaths, compared with 20.155 which would have been expected in the general Metropolitan Life Group. This is 64 per cent more than expected, or a ratio of 164. The males with moderately advanced tuberculosis showed a ratio of 238; those with far advanced disease, a ratio of 396. The females showed higher ratios: minimal, 222; moderately advanced, 806; and far advanced, 1,144 (table 5).

In general, the males in the groups with advanced tuberculosis showed higher mortality ratios in the age group less than forty-five; the females, in the age group less than thirty. In the groups with minimal disease, neither sex showed an appreciable decrease in ratios with increasing age.

Among the males, the excess mortality was due almost entirely to deaths from tuberculosis. This was not so pronounced among the females.

As mentioned above, some patients were lost from follow-up observations after varying lengths of time. Some letters from Mt. McGregor[5] went unanswered for various reasons. When a reply was not received directly from

[5] Follow-up was done by Miss Wesleyna Smith, Mt. McGregor Sanatorium.

the expatient, an attempt was made to get information from his former supervisor, his physician, or his co-workers. This was not always successful, especially during World War II, when so many people changed residence. It is not likely that all of the "lost" patients have died. However, assuming that each one died in the year in which he became a "lost case," the ratios of actual to expected deaths would have been:

	Males	Females
Minimal	228	632
Moderately advanced	302	1,151
Far advanced	448	1,144

Probably the mortality lies somewhere between these ratios and the ratios actually experienced, but nearer the ratios actually experienced.

SUMMARY

The experience is described of 1,213 Metropolitan Life Insurance Company employees who were first treated for pulmonary tuberculosis between 1919 and 1944 at the Company's Sanatorium at Mt. McGregor, New York, and were followed to 1949. The rate of relapse is shown for each year up to ten years after completion of treatment, also for the group followed at least five years and for the group followed at least ten years. The mortality after completion of first treatment was calculated by sex and stage of disease and was compared with mortality rates of Metropolitan Life Insurance Company Group Contract experience.

Two hundred and eighteen of the 1,213 patients who were first treated for tuberculosis at Mt. McGregor Sanatorium terminated their treatment by death. The percentage of deaths increased with the extent of the disease. The percentage of deaths was higher in the early years of admission to the hospital than in the later years.

Relapse of tuberculosis after completion of the treatment was lowest for those who had minimal tuberculosis. The rate of relapse at each stage of disease decreased with the number of years elapsed after completion of treatment.

The mortality of expatients increased with the increase in stage of disease. The mortality was higher for the women than for the men.

SUMARIO

Las Recaídas y la Mortalidad en la Tuberculosis Pulmonar

Descríbese lo observado en 1,213 empleados de la Compañía Metropolitana de Seguro de Vida, que fueron tratados por primera vez por tuberculosis pulmonar entre 1919 y 1944 en el Sanatorio de la Compañía en Mt. McGregor, Nueva York, y mantenidos en observación hasta 1949. Se presenta el coeficiente de recidivas para cada año hasta diez años después de terminar el tratamiento, y además en el grupo observado a lo menos cinco años y en el observado a lo menos diez años. Se calculó la mortalidad por sexo y por período de la

enfermedad después de terminar el primer tratamiento, comparándola con los coeficientes en el grupo de asegurados en la Compañía.

De los 1,213 enfermos tratados por primera vez por tuberculosis en el Sanatorio de McGregor, en 218 terminó el tratamiento con la muerte. El porcentaje de muertes aumentó según la extensión de la enfermedad, siendo más elevado en los primeros años de ingreso en el hospital que en los subsiguientes.

Las recaídas de la tuberculosis, después de terminar el tratamiento hospitalario, alcanzaron su cifra más baja en los que tenían tuberculosis mínima. El coeficiente de recidivas para cada período de la dolencia descendió conforme al número de años transcurridos después de terminar el tratamiento.

La mortalidad en los ex-enfermos aumentó al avanzar el período de la enfermedad, siendo más alta para las mujeres que para los hombres.

RESUME

Rechute et mortalité dans la tuberculose pulmonaire

Description d'une expérience portant sur un groupe de 1.213 employés de la «Metropolitan Life Insurance Company,» traités pour la première fois pour tuberculose entre 1919 et 1944 au sanatorium de cette compagnie à Mt. Gregor, Etat de New York, et qui ont été suivis jusqu'en 1949. Le taux de rechute est présenté pour chaque année jusqu'à dix ans après terminaison de la cure; il en est de même pour le groupe suivi pendant un minimum de cinq années et pour le groupe suivi pendant un minimum de dix années. Le taux de mortalité, à la suite de la première cure, a été calculé par sexe et par stade de la maladie puis comparé avec les taux de mortalité de l'expérience «Metropolitan Life Insurance Company Contract.»

Deux cent dix-huit des 1.213 malades dont le traitement initial pour tuberculose avait été effectué au sanatorium de Mt. Gregor ont succombé. Le pourcentage des décès s'accroissait selon le degré de la maladie. Le pourcentage des décès était plus élevé dans les premières années de l'hospitalisation que dans les années suivants.

Le nombre de rechutes après terminaison du traitement était le plus faible dans les cas de tuberculose *à minima*. Le taux de rechute à chaque stade de la maladie s'abaissait selon le nombre des années écoulées après terminaison de la cure.

Chez les «ex-patients» la mortalité s'accroissait avec le stade de la maladie. La mortalité était plus élevée chez la femme que chez l'homme.

SOME SERIOUS COMPLICATIONS OF TUBERCULOUS LYMPH NODES: FATAL HEMOPTYSIS, FALSE ANEURYSM, OCCLUDED CISTERNA CHYLI, AND OBSTRUCTIVE JAUNDICE[1]

A Review with a Report of Five Cases

GODFREY L. GALE

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INTRODUCTION

Tuberculous lymph nodes may cause a wide variety of complications (1). They may compress or rupture into the bronchus, trachea, esophagus, pericardium, pleura, lung, stomach, or bowel. They may erode blood vessels, with the production of a false aneurysm or with resulting hemorrhage or miliary dissemination of the disease. They may cause paralysis of the recurrent laryngeal nerve, the vagus, phrenic, or sympathetic trunk. In the operative removal of tuberculous nodes in the neck, various accidents may occur such as the loss of one or both accessory nerves (2). In fact, tuberculosis of the lymph nodes should always be regarded seriously, and patients should be afforded the benefit of adequate sanatorium care with antimicrobial drugs (3).

This paper presents the case reports of 5 patients treated in the Toronto Hospital for Tuberculosis, Weston, Ontario. All had serious complications from tuberculous lymph nodes, and 4 of the 5 died. All of the cases are unusual and 2 are of some rarity.

CASE REPORTS

Case 1. Fatal hemoptysis: I. L., a 39-year-old white man, was admitted to the Toronto Hospital for Tuberculosis on August 17, 1948. He gave a ten-month history of fatigue and recurring backache. Roentgenography showed active tuberculosis of the bodies of the ninth and tenth thoracic vertebrae, with destruction of the intervening disc and a large paravertebral abscess. The lung fields were clear apart from prominent left hilar shadows. Four days after admission he developed an acute paraplegia at the site of his spinal lesion. The paravertebral abscess was aspirated, and 20 cc. of heavy, greenish pus which was positive for tubercle bacilli on culture were recovered. His bladder was connected to tidal drainage. Decompression of the spine by costotransversectomy was being considered when he had a sudden overwhelming hemoptysis and died on the fourteenth day after his admission.

Autopsy showed a tuberculous extradural abscess compressing the spinal cord (figure 1). In the chest there was a mass of caseating tuberculous lymph nodes around the bifurcation of the trachea, with a traction diverticulum of the esophagus. One of these nodes had ulcerated into the left main bronchus, probably eroding a bronchial artery, with the resulting fatal hemoptysis.

Comment: The possibility of ulceration of a tuberculous lymph node into a bronchus had been known since Leblond's first description of it in 1824 (4). The first case to be reported in the United States, apparently, was by Scobie in 1934 (5), who listed an extensive bibliography and found 20 recoveries in 95 cases in

[1] From the Toronto Hospital for Tuberculosis, Weston, Ontario.

FIG. 1. *Case 1.* (I.L.) Sagittal section of thoracic spine showing compression of spinal cord by an extradural tuberculous abscess.

the literature at that time. In the past twenty years this condition has been increasingly recognized, and there have been more than 1,000 publications on this subject (1, 4–15). Some writers such as Schwartz believe that this is the dominant factor in the spread of tuberculosis of the lungs. He has records of 700 proved cases of ulceration of tuberculous lymph nodes into the bronchi in autopsies on patients who died with active pulmonary tuberculosis (14).

In the experience of the present writer, rupture of a tuberculous lymph node into a bronchus has not been a common autopsy finding, but it is periodically recognized at bronchoscopy and in bronchograms. At bronchoscopy, a shallow, gray ulcer may be noted with a central sinus leading to the lymph node beyond. Sometimes a button of granulation tissue presents with caseation at the center. Healing leaves a depressed gray scar, and this may cause quite a marked stenosis. A bronchogram in such cases may show a sinus tract or a depressed pocket, with

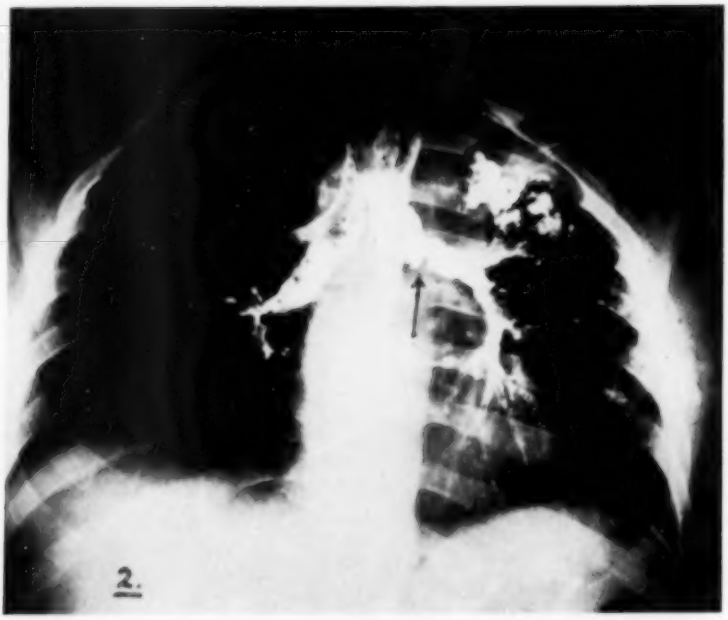

FIG. 2. Bronchogram showing a depressed scar in the medial wall of the left main bronchus where a tuberculous lymph node had ulcerated through. Immediately distal is a fibrous stricture. (On the medial side is Lipiodol® in the esophagus.) *See Case 1.*

or without stenosis of the affected bronchus. No bronchogram was taken in the above fatal case, but figure 2 is a bronchogram taken of another patient who had a bronchogenic spread of tuberculosis in the left lung. It shows a small crater where a lymph node had ulcerated into the medial wall of the left main bronchus, with a characteristic stricture adjacent to it. The crater and stricture in this case were also noted at bronchoscopy.

Ulceration of a lymph node into a bronchus may cause varied symptoms. It is not uncommon for patients to report coughing up fragments of calcareous material or even to produce them for inspection. A calcareous lymph node ulcerating into a bronchus may occasionally be removed at bronchoscopy or require urgent thoracotomy and removal for exsanguinating hemorrhage. A large caseous lymph node suddenly discharging into a bronchus may cause a fatal asphyxia, especially in a child. Pressure from enlarging hilar lymph nodes or the inflammatory reaction around them, even without ulceration through the bronchial wall, is a well-known cause of atelectasis of the middle lobe and occasionally of the lingula.

Case 2. Fatal hematemesis: M. C., a 69-year-old white woman, was admitted to the Toronto Hospital for Tuberculosis on December 6, 1938. One year prior to admission, an epithelioma of the left cheek had been treated by radium. On admission to the hospital, she had

widespread tuberculosis of the lymph nodes on both sides of the neck, including the supraclavicular regions, and in the left axilla. There were multiple discharging sinuses, some of which penetrated to the transverse processes of the cervical vertebrae. Pus from the sinuses was positive for tubercle bacilli on culture. In addition, she had bilateral, inactive, nonbacillary pulmonary tuberculosis and a nontuberculous cystitis and pyelonephritis. She was treated with bed rest and ultraviolet light, but her condition steadily deteriorated. The scar on the left cheek where she had had radium treatment broke down, perhaps as a result of the ultraviolet light. Early in August, 1939, she had several small hematemeses, and on August 20, 1939, she had a massive fatal hematemesis.

Autopsy showed the stomach and intestinal tract full of blood. The source of the hemorrhage was a small ulcer in the esophageal wall about the level of the cricoid cartilage, with caseation in its base. It lay at the termination of one of the open tuberculous sinuses in the neck, and it had eroded a varicose esophageal vein.

Comment: Tuberculous ulcers of the esophagus are uncommon but not unknown (1, 16–21). Coleman and Bunch analyzed 75 cases of acquired, nonmalignant esophagotracheobronchial communications and found that 14 of this series were due to tuberculosis (21). The source of the fistula is usually caseating or calcifying tracheobronchial nodes. Any case of traction diverticulum of the esophagus from tuberculous lymph nodes is a potential fistula. These fistulas should be investigated with Lipiodol ® rather than barium, and a useful test is to perform a bronchoscopy after putting methylene blue into the esophagus. Ono's sign is the appearance of rales in the lungs after swallowing water. Tuberculous esophagocutaneous fistulas, as in the present case, have also been described but are less common (17, 19). The modern treatment of these fistulas is to do a gastrostomy if a serious leak is present. If the fistula is small, however, there is a good prospect of its closing and healing with antimicrobial drugs alone. A large fistula may need to be excised after it has had sufficient time to become quiescent under a preliminary course of antimicrobial drugs.

Case 3. False aneurysm: B. M., a 15-year-old North American Indian girl, was admitted to the Toronto Hospital for Tuberculosis on November 30, 1945. On admission to the hospital, she had bilateral, moderately advanced, active pulmonary tuberculosis, and the sputum was positive for tubercle bacilli on culture. A serologic test for syphilis (Kahn) was negative. A right artificial pneumothorax was successfully induced, but on the left there were adhesions. As she was too young for a thoracoplasty, a left extrapleural pneumothorax was performed instead. She had several courses of streptomycin-PAS, with slow improvement in the pulmonary disease. She developed many extrapulmonary tuberculous lesions, however, including involvement of the right middle ear, the left sacroiliac joint, and four separate areas in the spine.

In February, 1951, a modified left thoracoplasty was performed to obliterate the extrapleural pneumothorax. This was successful, and she made a good convalescence from the operation. About the summer of 1950, it had been noted for the first time that there was a firm, rounded mass in the left upper quadrant of the abdomen near the costal margin. It was believed this might be an enlarged spleen from amyloidosis. On April 20, 1951, however, the patient had a sudden severe pain in the left upper quadrant of the abdomen and fainted. A few hours later she passed a large melena stool. Hemorrhage from the bowel continued and, in spite of a blood transfusion, she died three days later.

Autopsy showed the third part of the duodenum stretched over a mass 5 cm. in diameter which lay over the left side of the bodies of the third and fourth lumbar vertebrae but was not connected with them. The mass consisted of a cystic space filled with blood and organ-

FIG. 3. *Case 3.* (B. M.) Tuberculous false aneurysm opening into the abdominal aorta on the right and into the third part of the duodenum on the left. The aneurysm has been cut open and laid out flat.

ized thrombus. On one side it opened into the third part of the duodenum, and on the other side into the abdominal aorta through a small round hole, which may have represented the remains of one of the lumbar arteries. The aorta itself was healthy, there being no atheroma or arteriosclerosis (figures 3 and 4). A serologic test for syphilis (Kahn) was negative. Histologic section of the wall of the cystic mass showed chronic inflammatory tissue not diagnostic of tuberculosis. It was thought, however, that this was in fact a false aneurysm from a sloughing para-aortic lymph node which had eroded into the aorta at one place and into the third part of the duodenum at another. The remains of the lymph node had been lost in the expanding aneurysm.

Comment: A false aneurysm resulting from a sloughing tuberculous lymph node is quite uncommon, although there have been a number of cases reported in the literature (22–30). In Colt and Clark's series of 239 consecutive cases of caseous and calcareous abdominal lymph nodes from the Aberdeen Royal Infirmary, there was not a single case of false aneurysm (23). In Scott's series of 96 cases of abdominal aneurysm, only one was due to tuberculosis (26). Scott, Maxwell, and Grimes, however, found 21 cases of aneurysm of the abdominal aorta due to tuberculosis in the literature and added one which had ruptured into the stomach (27). The above case is probably the twenty-fifth abdominal, false aneurysm due to tuberculosis reported to date. The condition is hard to diagnose in life, and practically all end fatally. There seems to be a special tendency for this kind of false aneurysm to rupture into the intestinal tract, especially into the third part of the duodenum as in the above case. This tendency is not shared by aneurysms caused by syphilis, arteriosclerosis, or infected pyogenic emboli. It is noted that occasionally an aneurysm may result from a mycotic tuberculous abscess in the wall of the vessel.

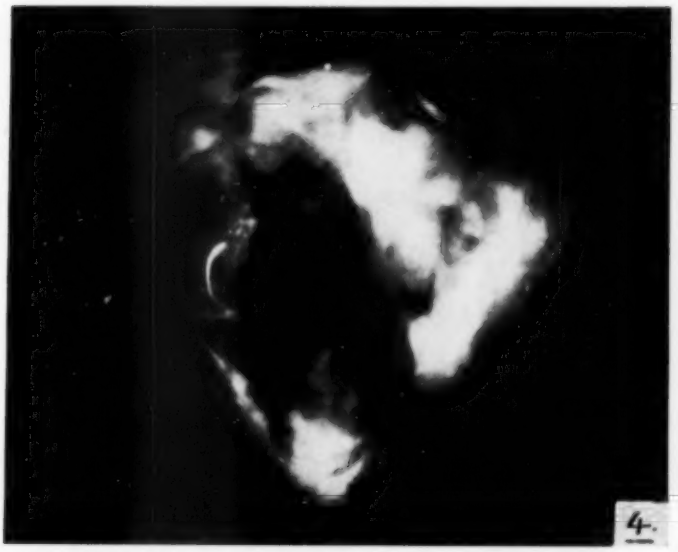

FIG. 4. *Case 3.* (B. M.) Roentgenogram of the false aneurysm showing areas of calcification (post-mortem specimen). The narrow crescent of dense calcification was seen in a roentgenogram of the abdomen, but its importance was not recognized.

Case 4. Occluded cisterna chyli: L. S., a 67-year-old white woman, was admitted to the Toronto Hospital for Tuberculosis on May 13, 1939. She was well, apart from tuberculous lymph nodes on the right side of the neck with discharging sinuses which were positive for tubercle bacilli on culture. There was no other demonstrable tuberculosis. She was treated by bed rest and ultraviolet light, and a tonsillectomy was done. The tonsils did not show any evidence of tuberculosis. In spite of treatment, the cervical adenitis steadily extended all over the right side of the neck, and many new sinuses appeared. On October 1, 1939, she had clinical evidence of a small myocardial infarction, although this was not confirmed by electrocardiogram.

In July, 1940, she complained of a cold abscess over the outer aspect of the right leg, and a roentgenogram showed a localized osteomyelitis in the shaft of the fibula (figure 5). Pus from this cold abscess grew tubercle bacilli on culture. In October, 1940, the middle three-quarters of the shaft of the right fibula was excised together with the surrounding tuberculous abscess in the soft tissues. This was followed by an exacerbation of the cervical adenitis, with spread to the right and left axillas and the appearance of fresh sinuses. The incision in the right leg failed to heal. Massive enlargement and caseation of the right inguinal nodes developed. Her condition gradually deteriorated, and she finally died six months after the operation without any acute episode or anything to suggest primary cardiac, renal, hepatic, or adrenal failure.

Autopsy failed to show any evidence of tuberculosis, apart from the lymphatic system and the unhealed incision in the right leg. There were massive enlargement and caseation of the lymph nodes on the right side of the pelvis and abdomen—inguinal, external iliac, common iliac, and para-aortic, right up to the cisterna chyli which was replaced by a mass of caseation. Some of the lymph nodes were several centimeters in diameter. Atherosclerosis of the aorta and the coronary arteries and a scar on the anterior wall of the left ventricle

FIG. 5. *Case 4.* (L. S.) Roentgenogram of the lower extremities showing tuberculous osteomyelitis of the shaft of the right fibula.

were noted. There was no evidence, however, of any recent myocardial damage. The lungs, liver, kidneys, and adrenals were not remarkable.

Comment: The operation on the right fibula had apparently opened fresh lymphatic channels, and she died of widespread tuberculosis of the lymphatic system of the right side of the pelvis and abdomen, culminating in the occlusion of the cisterna chyli. This is a rare condition. In the past thirty years, only one other case has been reported of tuberculous occlusion of the cisterna chyli. This was by Stacey Wilson in 1926 (31). His case was that of a 16-year-old girl who died of a slow wasting disease over a period of three years. At autopsy, the only lesion found was complete occlusion of the cisterna chyli by tuberculous granulation tissue. In addition to this case, three publications on tuberculosis of the thoracic duct have appeared in European journals (32–34).

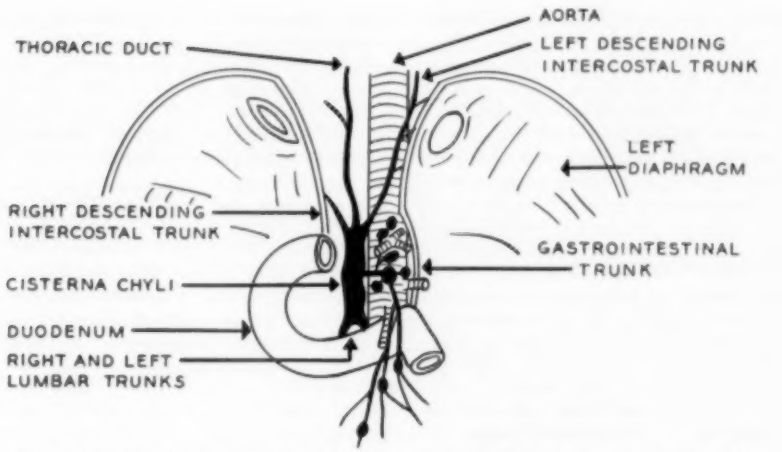

FIG. 6. The lymphatic connections of the cisterna chyli. *See Case 4* (after Netter)

The cisterna chyli is a small sac, one-third to one-half inch in diameter and two to three inches long. It lies on the right side of the bodies of the first and second lumbar vertebrae between the aorta and the right crus of the diaphragm. It usually has five tributaries: the gastrointestinal lymph trunk, which drains the celiac and the superior mesenteric nodes; a pair of lumbar lymph trunks, which drain the para-aortic nodes, large bowel, kidneys, adrenals, pelvis, lower abdominal walls, and lower extremities; and two descending intercostal lymph trunks, which drain the lower part of the chest. The cisterna chyli empties above into the thoracic duct which enters the right side of the posterior mediastinum through the aortic hiatus (figure 6). The thoracic duct may be ligated above the diaphragm without harmful effect as there are communicating channels below the diaphragm which can carry the chyle back into the circulation. But, if the cisterna chyli is occluded, then there appears to be great difficulty in establishing collateral channels, and the patient wastes away. It would be interesting to know how often the cisterna chyli is occluded by secondary carcinoma. Obstruction by Hodgkin's disease may cause enlargement of the lacteals and chylous ascites, neither of which were noted in the above case.

Case 5. Obstructive jaundice: S. T., a 23-year-old Japanese-Canadian man, was admitted to the Toronto Hospital for Tuberculosis on August 15, 1950. He gave a three-month history of enlarged lymph nodes on the right side of the neck, with abscess formation and a discharging sinus. The pus grew tubercle bacilli on culture. The chest roentgenogram was clear. A serologic test for syphilis (Kahn) was negative. He was given a one-hundred-day course of streptomycin, 0.5 gm. twice a day, and PAS, 14 gm. daily, which was the routine treatment at that time. In November, 1950, one month after the end of his course of streptomycin-PAS, he had a short attack of pyrexia, malaise, and anorexia followed by increasing jaundice with bile pigment in the urine and clay-colored stools. There was no abdominal pain. The blood count was normal; prothrombin time, 16 seconds (control, 15 seconds); van den Bergh direct, 3.5 mg. per 100 cc.; indirect, 5.0 mg. per 100 cc.; alkaline phosphatase,

8 K.A. units; cephalin cholesterol flocculation test, 1-plus. The serum alkaline phosphatase later rose to 129 K.A. units. The liver became enlarged, and the jaundice continued unchanged. It was clear that the patient was suffering from an obstructive jaundice, but the cause of this was not apparent until a firm mass became palpable in the right hypochondrium. The mass was smooth, rounded, fixed, and not especially tender. Roentgenographic examination of the abdomen did not reveal any calcification in the mass, but it was thought that it might perhaps be due to a group of tuberculous lymph nodes in the region of the porta hepatis obstructing the common bile duct.

Laparotomy, which was performed by Dr. F. G. Kergin, revealed this to be the case. The pylorus, first part of the duodenum, and head of the pancreas were pushed forward by a mass of enlarged lymph nodes half the size of a man's fist in which caseation could be seen. The gallbladder was thin-walled and distended. The common bile duct above the first part of the duodenum was distended to approximately 2.5 cm. in diameter. No attempt was made to excise the mass of nodes, but the common bile duct was anastomosed to the duodenum. A liver biopsy showed biliary cirrhosis. Following the operation, the jaundice rapidly cleared. The patient was given a second course of streptomycin-PAS. With this he made an uninterrupted recovery, and the mass of nodes in the abdomen became smaller until they were no longer palpable. Liver function tests returned to normal, and he was discharged in good health in August, 1951.

Comment: Obstructive jaundice is often an interesting problem, and pressure from tuberculous lymph nodes in the region of the porta hepatis is usually—and rightly—mentioned in the differential diagnosis. In actual fact it must be one of the rarest conditions in surgery. A careful search of the literature over the past thirty years has failed to reveal a single case reported in the English journals. The only reference in English is a brief abstract from a French journal in the J.A.M.A. of 1925 (35). Colt and Clark's series of 239 consecutive cases of caseous and calcareous abdominal lymph nodes did not have a single case of obstructive jaundice (23). There have been two articles on roentgenographic deformity of the duodenum from pressure of enlarged lymph nodes (with one case due to tuberculosis), but in neither article was jaundice reported (36, 37). In fact the only cases of this condition reported in the literature have been in European

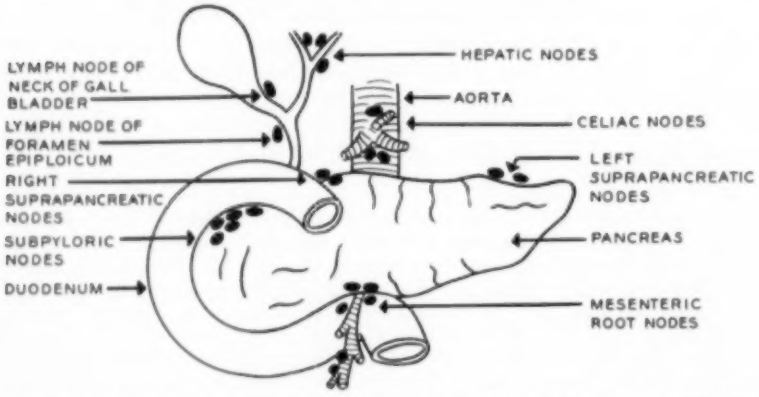

FIG. 7. Lymph nodes in the region of the porta hepatis and the common bile ducts. *See Case 5* (after Netter).

journals (38–44), including the one abstracted in the J.A.M.A. in 1925 (38). In this last article, six cases had been collected from the literature and a seventh added. There must have been cases which never reached the literature but, at best, this must be a very rare cause of obstructive jaundice. It appears to be a pathologic curiosity rather than a matter of actual surgical importance.

There were several groups of lymph nodes around the first part of the duodenum and common bile duct (figure 7). It is a little hard to be sure how the infection reached these lymph nodes in the above case, but there are two possibilities. It may have traveled up the lacteals from the region of the cecum, or it could have come from the lower part of the pleural space on either side along lymphatics which drain down into the para-aortic lymph nodes lying on the bodies of the twelfth thoracic and first lumbar vertebrae (45). There was no history of pleurisy and no roentgenographic evidence of pulmonary tuberculosis, but that does not exclude this latter possibility.

The response to streptomycin–PAS therapy is fairly typical. In 1950, it was not realized how important it is to give long-term chemotherapy for tuberculous lymph nodes, and the hundred days of treatment received were only sufficient to make the infection quiescent without obtaining healing. The flare-up of disease took place one month after the end of this course of treatment. After the laparotomy when the diagnosis had been established, the second course of streptomycin–PAS completed the cure.

SUMMARY

Five cases of serious complications of tuberculous lymph nodes are reported, 4 of them fatal. The fatal cases were: (*1*) fatal hemoptysis from erosion of a bronchial artery; (*2*) fatal hematemesis from erosion of a varicose esophageal vein; (*3*) fatal hemorrhage from a false aneurysm, which opened into the abdominal aorta on the one side and into the third part of the duodenum on the other; (*4*) fatal occlusion of the cisterna chyli by tuberculous granulation tissue.

The fifth case was one of obstructive jaundice from pressure on the common bile duct by tuberculous nodes in the region of the first part of the duodenum.

The modern treatment of tuberculous lymph nodes by long-term antimicrobial drugs should result in fewer serious complications.

SUMARIO

Algunas Complicaciones Graves de los Ganglios Linfáticos Tuberculosos: Hemoptisis Letal, Hematemesis Letal, Seudoaneurisma, Oclusión de la Cisterna de Pecquet e Ictericia Oclusiva

Preséntanse 5 casos de complicaciones graves de la tuberculosis de los ganglios linfáticos, 4 de ellos letales. Los casos letales fueron: hemoptisis letal debida a erosión de una arteria bronquial; hematemesis letal debida a desgaste de una vena esofágica varicosa; hemorragia letal procedente de un falso aneurisma que se abría por un lado en la aorta abdominal y por el otro en la tercera porción del duodeno; oclusión letal de la cisterna de Pecquet por tejido de granulación tuberculoso.

El quinto caso era de ictericia oclusiva debida a compresión del colédoco por ganglios tuberculosos en la región de la primera porción del duodeno.

El tratamiento moderno de la tuberculosis ganglionar con drogas antimicrobianas administradas a largo plazo debe dar por resultados menos complicaciones graves.

RESUME

Quelques complications graves des ganglions lymphathiques tuberculeux: Hémoptysie fatale, hématémèse fatale; faux anévrisme, occlusion de la citerne de Pecquet et ictère par obstruction

L'auteur rapporte cinq cas de complications graves provoquées par des ganglions lymphatiques tuberculeux dont quatre ont été fatales. Les cas mortels étaient: une hémoptysie fatale due à l'érosion d'une artère bronchique; une hématémèse fatale due à l'érosion d'une varice oesophagienne; une hémorragie fatale due à un faux anévrisme qui s'est ouvert dans l'aorte abdominale d'un coté et dans la troisième partie du duodénum de l'autre. Une occlusion fatale de la citerne de Pecquet par un tissu de granulation d'origine tuberculeuse.

Le cinquième cas était un ictère par compression du cholédoque dû à la présence de ganglions tuberculeux au niveau de la première partie du duodénum. Le traitement moderne des ganglions lymphatiques tuberculeux par la thérapie anti-microbienne de longue durée devrait entraîner une réduction des complications graves.

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CORTISONE, CORTICOTROPIN, AND ANTIMICROBIAL THERAPY IN TUBERCULOSIS IN ANIMALS AND MAN[1]

A Review

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INTRODUCTION

Corticotropin (ACTH) and cortisone have been used experimentally in the treatment of infectious disease (1–4) with marked reduction in fever and toxicity, but the underlying disease processes in most instances have followed their usual course in spite of the patients' apparent well-being. Complications and progression of disease have occurred as in the untreated patient and are perhaps even more frequent in the hormone-treated person. The major effect of these drugs in infections appears to be a suppression of the usual inflammatory response of the body with impairment of granulation tissue formation, macrophage activity, and fibroblastic repair (5–7). Because of the effect of depleting the lymphocytic reserve, antibody formation may be depressed somewhat during long-term therapy, but most workers believe that the adrenal steroids do not act directly on antigen-antibody mechanisms except in a nonspecific manner by limiting the inflammatory response (5). As the inflammatory, macrophagic, and fibroblastic responses are necessary in localizing the disease process, destroying the infecting agent, and repairing the damaged tissues, corticotropin and cortisone have been regarded in general as contraindicated in infectious disease. When infection has been a complication of a disease in which adrenal steroid therapy seems indicated, the concurrent administration of appropriate antimicrobials has been advised (8).

A few writers have recommended that corticotropin or cortisone be used in the specific treatment of infections, especially in overwhelming toxic states in which temporary improvement in the patient's symptoms is imperative, e.g., pneumococcal pneumonia, peritonitis, meningitis (9), and typhoid fever (4), or when inhibition of excessive granulation tissue and fibrosis is desirable, e.g., tuberculous meningitis (9–12), eye inflammations (13), and pelvic inflammatory disease (14). However, all of these writers emphasize that adrenal steroids are to be used in this manner only in conjunction with appropriate intensive antimicrobial therapy.

Of all infectious diseases, tuberculosis is probably the one in which corticotropin or cortisone therapy is regarded as most hazardous. Active tuberculous infection is considered one of the usual contraindications of adrenal steroid use and even an absolute contraindication by some authorities (15, 16). The Committee on Therapy of the American Trudeau Society has stated that, when the use of corticotropin or cortisone is necessary in the presence of active tuberculosis, streptomycin–PAS should likewise be used (8).

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The exact status of steroid therapy in tuberculous disease is far from clear, however, and the following questions remain unanswered: How detrimental is corticotropin or cortisone therapy for tuberculosis in humans? Will antituberculous chemotherapy protect a patient against any deleterious effects of corticotropin or cortisone? Finally, does combined adrenal steroid-antimicrobial therapy have something to contribute to the treatment of tuberculosis? In the hope of clarifying these questions, an attempt has been made to assemble a comprehensive review of the literature on the subject.

THE USE OF CORTICOTROPIN OR CORTISONE WITHOUT ANTIMICROBIALS IN EXPERIMENTAL TUBERCULOSIS

Adverse influence of cortisone: Experimental studies on tuberculous animals indicate that cortisone has a deleterious effect. Spain and Molomut (6) found that cortisone caused tuberculous lesions in the guinea pig to become "more extensive, more widely distributed, and less well localized" than in controls. Bloch, Vennesland, and Gurney (17) found greater involvement and described increased numbers of organisms in cortisone-treated animals. However, they found no significant alteration in the nature of the tissue response histologically; nor, when 55 days elapsed between treatment and autopsy, was the extent of disease greater than in the controls. Albino rats do not ordinarily succumb to virulent tubercle bacilli, but, when treated with cortisone, Michael, Cummings, and Bloom (18) found they developed diffuse lesions with decreased inflammatory response and died. In another study, Cummings, Hudgins, Whorton, and Sheldon (19) found only small foci and no caseation in control albino rats; but, in cortisone-treated animals, 8 of 10 died with marked bronchogenic spread, the extensive lesions displaying practically no fibroblastic activity. There was also evidence of secondary pyogenic pneumonitis in the latter animals. Hart and Rees (20) observed that cortisone produced increased susceptibility to tuberculosis in the mouse. Solotorovsky, Gregory, and Stoerck (21) confirmed this finding and also demonstrated that cortisone cancelled the protective action of vaccination in mice infected with virulent bacilli.

Lurie, Zappasodi, Dannenberg, and Swartz (22) noted a mixed response of the tuberculous rabbit to cortisone. There were more tubercles and more caseation necrosis with extraordinarily large numbers of organisms; but there were reduced size and dissemination of tubercles, less involvement of draining lymph nodes, and increased phagocytic activity. It was concluded that cortisone made these genetically susceptible rabbits react more like natively resistant rabbits.

Adverse influence of corticotropin: Bunn and Drobeck (23) have reported harmful effects of corticotropin and cortisone on rabbit eye tuberculosis. Corticotropin given two weeks after infection was produced resulted in a more rapid progression of disease, greater destruction of tissues, and frequent metastatic lesions. If the drug was discontinued after only two weeks of administration, however, the subsequent course of the disease was approximately the same as in the controls, with no significant difference after three months. When corticotropin was given before inoculation, no lesion developed until it was discontinued, after

which rapid destruction of the eye followed. When given six months after disease was established, it had no apparent effect except to increase susceptibility to secondary pyogenic invaders.

It should be emphasized that these studies of the rabbit eye represent the only instance in which corticotropin has been shown to be detrimental to any animal species with tuberculosis. Whether this means corticotropin is less hazardous than cortisone in tuberculosis in man is not clear.

The relative lack of evidence incriminating corticotropin as compared with cortisone may be due to fewer studies with this substance, to differences in physiologically equivalent doses of the drugs in different species, or to variations of the frequencies of administration and duration of actions of the two hormones. LeMaistre and Tompsett (24) discussed this question in a study in which they demonstrated lesions grossly, although not histologically, resembling tuberculosis due to infections with *C. pseudotuberculosis murium* which emerged in cortisone-treated animals but not in rats which received corticotropin.

Cortisone or corticotropin studies in which results were not necessarily adverse: The preceding studies comprise the major evidence of adverse effects of corticotropin and cortisone on experimental tuberculosis. Other workers have failed to confirm some of these findings. LeMaistre and Tompsett (25) studied experimental tuberculosis in the guinea pig, using large doses of both corticotropin and cortisone separately, and reported no evidence of enhancement of the tuberculous process with less involvement than controls after fourteen days of treatment and no significant difference in extent of lesions or numbers of tubercle bacilli at twenty-eight days. Karlson and Gainer (26) treated tuberculous guinea pigs with cortisone daily for 62 days and found no significant enhancement of disease as compared with control animals. Bernard and Kreis (27) compared 4 guinea pigs treated with cortisone for thirty-nine to fifty days with 10 controls and reported no exacerbation, the course and pathologic extent of the lesions being equivalent. Recently Weimer and associates (28), in a very thorough study involving larger numbers of guinea pigs than had been used before, found that treatment with 0.1, 1.0, and 10.0 mg. of corticotropin daily and with 0.2, 2.0, and 20.0 mg. of cortisone daily for twenty-one days was without significant effect on the extent of tuberculosis in animals autopsied sixty-eight days after termination of treatment. Bacos and Smith (29) gave corticotropin to partially immunized rabbits throughout one hundred and twenty days of infection and found progressive disease developed, but this was of less extent than in the controls.

It is interesting to note the number of studies in experimental tuberculosis which indicate no adverse action of corticotropin in comparison with the single study (23) which did.

Ebert (7) studied the effect of cortisone on experimental tuberculosis by using the rabbit ear chamber technique, and demonstrated decreased vascular damage and better vascular tone with decreased exudation of cells and fluid. He predicted, therefore, that disease states would be affected according to the usefulness of the inflammatory reaction. In tuberculosis, in which the usefulness is ap-

parently mixed, treatment may be rewarded with less tissue destruction, but also may be complicated by poorer localization and increased multiplication of organisms.

THE USE OF CORTICOTROPIN OR CORTISONE WITHOUT ANTIMICROBIALS IN HUMAN TUBERCULOSIS

In addition to the studies in experimental animals, there are reports in the literature in which 20 patients with active pulmonary tuberculosis were treated with corticotropin and/or cortisone without antituberculous drugs. Freeman and associates (30) treated 2 patients with corticotropin and, although both became afebrile and asymptomatic, there was evidence by roentgenography of increased extent of disease while under treatment. Thorn and co-workers (13) described a patient of Favour's treated with corticotropin; again symptomatic relief resulted, but exacerbation of disease occurred following therapy. LeMaistre and associates (31, 32) treated 4 patients with corticotropin and 3 with cortisone for one to four weeks. All had advanced pulmonary and laryngeal tuberculosis. Five of the 7 patients showed no significant improvement or deterioration, and 2 died during therapy, but without evidence of adverse hormonal effects on the tuberculous process. All became nearly asymptomatic within six to seventy-two hours of onset of treatment and showed rapid healing of laryngeal ulceration. Comden and Netzer (33) reported 3 patients with far advanced tuberculosis and rheumatoid arthritis to whom corticotropin was given for long periods. All were improved symptomatically, but one showed progression of disease by roentgenography. The others showed no change by roentgenography after three and a half to six months of treatment. Traut and Ellman (34) described a patient with a marked increase of supposedly inactive tuberculosis after three months of cortisone given for rheumatoid arthritis. At the same time they stated that other patients with inactive pulmonary tuberculosis who received cortisone for arthritis experienced no increase in their disease. Finally, Wallner, Thompson, and Lichtenstein (35) gave corticotropin or cortisone for ten to twenty-two days to 6 patients with severe pulmonary and laryngeal tuberculosis. Again, all had symptomatic relief, but no significant change in laryngeal ulcerations resulted. Four showed no change, and 2 showed improvement on chest roentgenograms.

In summary, of 20 patients with known active tuberculosis receiving corticotropin or cortisone, 2 were improved, 11 were unchanged, and 7 were worse after treatment.

In August, 1952, 81 chiefly unpublished cases of persons treated with cortisone and/or corticotropin in whom tuberculous infection was present were summarized (8) for apparent effect on the tuberculosis as follows: 10 favorable, 25 no effect or doubtful effect, and 46 unfavorable. Although details about the extent of the disease, treatment, and criteria of evaluation were lacking, the Committee on Therapy of the American Trudeau Society concluded: (*1*) in the presence of active tuberculosis, corticotropin and cortisone therapy is at least potentially hazardous; (*2*) associated inactive tuberculosis should be evaluated well before beginning therapy for other conditions; (*3*) even in the apparent absence of

tuberculosis, roentgenograms should be taken before and after courses of these drugs; and (*4*) when treatment with them is indicated, even in the presence of active tuberculosis, such therapy should be combined with streptomycin–PAS.

More drastic evidence of the enhancement of tuberculosis by corticotropin or cortisone are the case reports describing the appearance of fulminating pulmonary or disseminated disease during or shortly after a course of these drugs in persons in whom tuberculosis was unsuspected previously. Thirteen such reports have been found. These are difficult to evaluate because it is not known in many of them what the status of the tuberculosis was before treatment was started (table 1).

For example, 2 of the 13 patients probably had active disseminating tuberculosis before receiving steroids and 3 had previously known clinical tuberculosis which also may have been active before treatment. Two of 3 in whom the clinical tuberculosis was apparently new were not reported as having pretreatment roentgenograms and, in the third case, although the patient was being treated for Weber-Christian disease, the pretreatment biopsy of a subcutaneous nodule was later regarded by some pathologists as representing tuberculosis. Certainly, however, in spite of the possibility of previously active disease, these case reports are strong evidence of the exacerbation of tuberculosis in some instances by corticotropin or cortisone therapy. It is not to be assumed, however, that all patients with tuberculosis will react adversely to these drugs.

THE USE OF CORTICOTROPIN OR CORTISONE WITH ANTIMICROBIALS IN EXPERIMENTAL TUBERCULOSIS

The next question is will antimicrobial therapy protect the tuberculous animal from adverse effects of corticotropin or cortisone. Unfortunately, there are few studies available. Spain and Molomut (6) found that tuberculous guinea pigs treated with streptomycin and cortisone demonstrated much less tuberculous involvement than control animals, but had more involvement than guinea pigs treated with streptomycin alone. In a second study (46), they showed that very small doses of cortisone combined with subeffective doses of dihydrostreptomycin resulted in more tuberculous involvement than when the same dose of dihydrostreptomycin was used alone, but again less than in the controls. In the study of Karlson and Gainer (26) with guinea pigs, the administration of cortisone combined with streptomycin appeared to have prevented restriction and healing of the tuberculous process. Therefore, although cortisone did not nullify streptomycin effectiveness in these experiments, the effect of the streptomycin was decreased in spite of a failure to demonstrate cortisone enhancement of tuberculosis in the guinea pigs which received no streptomycin. Bloch, Vennesland, and Gurney (17) demonstrated that streptomycin did not completely protect the guinea pig from the ill effects of the cortisone when treatment was begun at the time of the inoculation; however, when it was begun twenty-four days after infection was produced, the streptomycin-cortisone–treated animals had even less involvement than those treated with streptomycin alone.

In animals other than the susceptible guinea pig, the results were consistently

TABLE 1

CASES IN WHICH ACTIVE TUBERCULOSIS APPEARED DURING OR SHORTLY AFTER CORTICOTROPIN OR CORTISONE THERAPY FOR NONTUBERCULOUS DISEASE

Reference	Disease Treated	Drug	Tuberculous Complication	Relationship of Tuberculosis to Treatment				Remarks
				New Disease	Apparently New Disease	Previous Inactive Tuberculosis	Previous Active Tuberculosis Likely	
Bogen (36)	Rheumatoid arthritis	Corticotropin	Pulmonary	×				Negative chest roentgenogram before treatment; flared after treatment
Bogen (36)	Rheumatoid arthritis	Corticotropin	Pulmonary			×		Inactive two years with "negative" sputum and pneumothorax; flared after treatment
Bogen (36)	Polyarteritis nodosa	Corticotropin	Lymphadenitis		×			Lymphadenopathy with biopsy diagnosis of polyarteritis; after treatment, biopsy diagnosis was tuberculosis
Kleinschmidt (37)	?	Cortisone	Miliary				×	Four-month illness with fever and pleural effusions; treated as polyarteritis, but only miliary tuberculosis found at autopsy
Fred (38)	Rheumatoid arthritis	Corticotropin-cortisone	Pulmonary	×				Negative chest roentgenogram before treatment; flared after treatment
Popp (39)	Rheumatoid arthritis	Cortisone	Pulmonary			×		Apparently arrested, but no roentgenogram just prior to treatment; flared after treatment
King (40)	Rheumatoid arthritis	Cortisone	Pulmonary	×				Developed pneumonitis, cavitation, and died; no bacteriologic confirmation; no autopsy
Doerner (41)	Weber-Christian disease	Cortisone	Meningeal		×			Negative roentgenogram before treatment; developed meningitis after; prior biopsy possibly tuberculosis

Harris-Jones (42)	Lupus erythematosus	Cortisone	Miliary			×		Pleurisy with effusion two years before treatment; miliary tuberculosis developed during treatment
Capon (43)	Scleroderma	Cortisone	Osseous		×			No chest roentgenogram prior to treatment; flared after treatment
Capon (43)	Lupus erythematosus	Cortisone	Miliary-meningeal				×	Pneumonitis, cough, sputum, roentgenographic changes prior to treatment; sputum studies negative for acid-fast bacilli
Walker (44)	Lupus erythematosus	Corticotropin	Miliary	×				Negative chest roentgenogram before treatment; flared during treatment
Davidson (45)	Lupus erythematosus	Corticotropin-cortisone	Miliary	×				Roentgenograms, sputum, gastric washings negative before treatment; flared during treatment

favorable. Cummings and associates (19) reported that streptomycin protected the albino rat from the harmful effects of cortisone. Thus, they postulated that humans, being variable in native resistance to tuberculosis, may respond to steroid therapy either like the guinea pig or like the albino rat, depending on their individual susceptibility to the tubercle bacilli.

The only study in the entire group involving corticotropin was that of Bacos and Smith (29) with partially immunized rabbits, and they found somewhat better results with corticotropin and dihydrostreptomycin than with dihydrostreptomycin alone.

Thus, there is no direct evidence that corticotropin when used with antimicrobials exerts any adverse effects on experimental tuberculosis in animals, and it is only the highly susceptible guinea pig in which cortisone was found to decrease the effectiveness of streptomycin.

THE USE OF CORTICOTROPIN OR CORTISONE WITH ANTIMICROBIALS IN HUMAN TUBERCULOSIS

Combined steroid-antimicrobial therapy for the treatment of human tuberculosis per se has been reported by a few writers. Kinsell (9) expressed the opinion in September, 1951, that such combination therapy would eventually have a place in the treatment of tuberculosis and mentioned 2 cases of tuberculous meningitis treated successfully in this manner at his clinic. Shane, Clowater, and Riley (10) pointed out that patients with tuberculous meningitis treated with streptomycin usually die, not of tuberculous toxemia, but of mechanical obstruction to cerebrospinal fluid circulation by exudate and granulation tissue. As steroid therapy would be expected to inhibit such granulations and fibrosis, they suggested a trial of cortisone in desperate cases. They described a patient treated with streptomycin intramuscularly and intrathecally and with para-aminosalicylic acid orally in whom cerebrospinal fluid block developed. This was unrelieved by streptokinase-streptodornase, and the patient became moribund. At this point, cortisone was added to the chemotherapy program, with remarkable symptomatic relief, complete alleviation of the block by two months, and restoration of normal cerebrospinal fluid after three months of combined therapy. Recently Shane and Riley (11) have reported 6 more patients with tuberculous meningitis treated with combined cortisone-antimicrobial therapy without intrathecal streptomycin, 4 of whom have recovered. They believe the use of cortisone in active tuberculosis to be a safe procedure if adequate antimicrobial therapy is employed. An editorial (12) commenting on this paper approved further clinical trials of combined therapy in view of the apparent safety of the treatment, but regarded it as a highly experimental procedure only to be performed under controlled conditions.

Meyer, Kinsell, and Parsons (47) attempted to prepare 3 patients with far advanced tuberculosis with extensive tracheobronchial involvement for surgery with corticotropin and streptomycin–PAS. Each patient developed a marked sense of well-being with nearly complete relief from cough, fever, and other symptoms. Two of the 3 patients showed marked improvement in the ulcerative tracheobronchitis, but in one this was only temporary despite continued treat-

ment and in the other there was progression of pulmonary disease. Pathologic examination of resected tissue in both cases revealed persistent active bronchitis and much parenchymal caseation with no surrounding fibrosis and little or no epithelioid granulation tissue. In the third case, there was no demonstrable effect on the laryngeal or pulmonary tuberculosis.

Houghton (48) recently reported he had used corticotropin in 18 patients with active tuberculosis who were receiving streptomycin–PAS. Three additional patients had severe hypersensitivity reactions to streptomycin or PAS. In 8 cases corticotropin permitted continued streptomycin-PAS therapy despite hypersensitivity reactions to one or both drugs. After four weeks of treatment, corticotropin could be withdrawn from 6 of the 8 patients who were found no longer to be hypersensitive to the antimicrobials. In 5 cases, gastrointestinal intolerance to PAS was relieved by very small doses of corticotropin; and, in 5 cases of progressive disease despite streptomycin-PAS therapy, corticotropin was added. In none of the cases were adverse clinical or roentgenographic effects seen during or following the administration of corticotropin. On combined therapy, severely ill patients were said to have improved very much and their progress was maintained after the withdrawal of corticotropin. Clinical and roentgenographic improvement was noted in some of the patients who had previously not responded to streptomycin or PAS alone.

An interesting case of the present writers (49) was a patient critically ill of disseminated lupus erythematosus with coexisting far advanced pulmonary tuberculosis. After one month of streptomycin–PAS, it was necessary to add corticotropin because of the increasing severity of the lupus. This responded immediately, and the patient became essentially asymptomatic with respect to both diseases. Within eight weeks of the start of hormone therapy, there were marked resolution of bilateral exudative disease, spontaneous closure of a large cavity, and "conversion" of sputum. The corticotropin was given in doses of 100 mg. per day at first, then 60 to 40 mg. for four months followed by three months of slowly decreasing dosage. Both the lupus erythematosus and tuberculosis have been inactive for more than eighteen months.

GENERAL SUMMARY

Corticotropin and cortisone, when used in infections, have been reported to limit inflammation, granulation, and fibrosis with improvement in symptoms, but often with complicating progression and dissemination.

In experimental tuberculosis in animals, deleterious effects have been demonstrated when cortisone and, in one instance, when corticotropin were used, but recent studies in the guinea pig have failed to confirm significant adverse effects; and, therefore, further studies are advisable. In view of the relative lack of evidence of enhancement of experimental tuberculosis by corticotropin as compared with cortisone, corticotropin may be the safer hormone. It is possible that differences of dosages, frequencies of administration, duration of action, and varying responses in different species may be the explanation for this apparent difference in the two drugs.

Of 20 patients with active tuberculosis who received corticotropin or cortisone

without antimicrobial coverage, 2 were better, 11 were unchanged, and 7 were worse after treatment. In addition, 13 instances of fulminating pulmonary or disseminated tuberculosis have been reported in patients with nontuberculous disease under treatment with corticotropin or cortisone. A few of these, however, may have had undiagnosed active tuberculosis before treatment.

In most experiments, combining streptomycin with cortisone has overcome the deleterious effects of the latter in guinea pig tuberculosis; however, cortisone did appear to decrease the effectiveness of the streptomycin in this species. So far as is known, combined corticotropin-streptomycin treatment has not been studied in tuberculous guinea pigs, and studies with corticotropin-isoniazid would also be of great interest. Streptomycin was found to afford the albino rat and partially immunized rabbits complete protection from harmful effects of cortisone and corticotropin administration, respectively.

Combination corticotropin or cortisone with streptomycin therapy has been employed in 31 known cases of active tuberculosis without ill effect and often with apparent improvement of the tuberculosis beyond that expected with streptomycin alone. The indications were tuberculous meningitis, tuberculous laryngitis, poorly responding pulmonary tuberculosis, streptomycin or PAS hypersensitivity, and coexisting disseminated lupus erythematosus and pulmonary tuberculosis.

Because no exacerbations have occurred in human tuberculosis with combined therapy, it would seem that the presence of active tuberculosis becomes a relative rather than an absolute contraindication to therapy with cortisone or corticotropin for nontuberculous conditions, providing effective antimicrobials are given concurrently. With more data, active tuberculosis eventually may be no contraindication at all. The possibility exists, however, that, even if streptomycin does prevent cortisone or corticotropin from aggravating the patients' tuberculosis, the hormones may result in some decrease in the effectiveness of the antimicrobial.

Although promising, the use of combined corticotropin or cortisone and antimicrobials in the treatment of certain types of tuberculosis per se is probably still to be considered an experimental procedure to be performed only under controlled conditions.

In chronic debilitating disease, such as rheumatoid arthritis or lupus erythematosus, when prolonged cortisone or corticotropin therapy is employed, the development of fulminating or disseminated tuberculosis is a possibility and chest roentgenograms should be obtained at regular intervals.

SUMMARY

An attempt has been made to review what is known of the effects of cortisone and corticotropin with and without antimicrobials upon tuberculosis in experimental animals and in humans.

There is convincing evidence, in certain experiments or cases at least, that cortisone and, to a lesser extent, corticotropin have adversely affected tuberculosis both in experimental animals and in man.

On the other hand, the bulk of evidence available to date indicates that the concurrent use of streptomycin will protect animals and man from deleterious effects of cortisone and corticotropin.

The combination of corticotropin or cortisone with streptomycin has been used in 31 cases of active tuberculosis without ill effect and often with accelerated improvement of the tuberculous disease.

On the basis of this review, it would appear that combined therapy, when needed, is a relatively safe procedure; but there is insufficient evidence to justify anything less than caution and careful observation when corticotropin or cortisone, even with streptomycin, are employed in the treatment of tuberculosis.

SUMARIO

La Cortisona, la Corticotropina y la Terapéutica Antimicrobiana en la Tuberculosis de los Animales y del Hombre: Repaso

Trátase aquí de repasar nuestros conocimientos de los efectos de la cortisona y la corticotropina (HACT) sobre la tuberculosis en los animales de experimentación y en los seres humanos, con y sin productos antimicrobianos.

Existen pruebas convincentes, a lo menos en ciertos experimentos o casos, de que la cortisona y, en menor grado, la corticotropina han afectado adversamente la tuberculosis, tanto en los animales de experimentación como en el hombre.

Por otra parte, el volumen de los datos disponibles hasta ahora indica que el empleo concurrente de la estreptomicina protegerá a los animales y al hombre contra los efectos nocivos de la cortisona y la corticotropina.

La combinación de corticotropina o cortisona con estreptomicina fué usada en 31 casos de tuberculosis activa sin efectos contraproducentes y a menudo con mejoría acelerada de la afección tuberculosa.

A base de esta reseña, parece que la terapéutica combinada, cuando se necesita, es un procedimiento relativamente inocuo; pero los datos son insuficientes para justificar cuanto no sea cautela y observación cuidadosa al emplear corticotropina o cortisona, aun con estreptomicina, en el tratamiento de la tuberculosis.

RESUME

Cortisone, Corticotrophine et thérapie antimicrobienne dans la tuberculose chez l'animal et chez l'homme: Compte-rendu

Les auteurs ont tenté de passer en revue les notions relatives aux effets de la cortisone et de la corticotrophine (ACTH) sur la tuberculose de l'animal en expérience et de l'homme, lorsqu'elles sont associées ou non aux antimicrobiens.

On possède des preuves convaincantes, tout au moins dans certaines expériences ou dans certains cas, que la cortisone et, à un moindre degré, la corticotrophine, aggravent la tuberculose chez l'animal en expérience et chez l'homme.

D'autre part, l'ensemble des données disponibles à ce jour indique que l'administration concomitante de streptomycine protègera les animaux et l'homme contre les effets pernicieux de la cortisone et de la corticotrophine.

L'association de la corticotrophine ou de la cortisone avec la streptomycine a été utilisée dans 31 cas de tuberculose en activité sans résultats défavorables et, souvent, avec une accélération de l'amélioration de la tuberculose.

Selon ce compte-rendu, il semble qu'en cas de besoin, le traitement combiné constitue un mode de traitement relativement sûr; toutefois, les preuves manquent pour justifier une attitude autre que prudente ou un manque de surveillance du malade quand la corticotrophine ou la cortisone, même avec adjonction de streptomycine, sont utilisées dans le traitement de la tuberculose.

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THE PREVALENCE OF STREPTOMYCIN-RESISTANT TUBERCLE BACILLI AMONG 5,526 PATIENTS ADMITTED CONSECUTIVELY TO HOSPITALS[1,2]

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INTRODUCTION

In order to determine the prevalence of streptomycin-resistant tubercle bacilli in an adult population group, a screening test for susceptibility of tubercle bacilli to streptomycin was performed on sputum or gastric specimens obtained from every patient admitted to the Tuberculosis Service of thirty Veterans Administration hospitals. This study was carried out during a seven-month period between May and December, 1952.

MATERIALS AND METHODS

The tests were performed by the "direct method," using a single tube of American Trudeau Society or modified Löwenstein culture medium containing 10 γ of streptomycin per ml. for comparison with the control tube containing no drug (1). Growth in the drug-containing tube which was equivalent to the control tube was considered to indicate resistance of the tubercle bacilli to streptomycin. No growth in the drug-containing tube or growth which was less than that in the control tube was considered as indicating susceptibility of the tubercle bacilli to streptomycin.

RESULTS

There were 5,526 persons "screened" by this method, of whom 2,993 gave a history of previous streptomycin therapy. Of the previously treated group, tubercle bacilli were cultured from 1,617 persons. Streptomycin-susceptibility determinations were reported for 1,360 of these strains. Tubercle bacilli completely resistant to 10 γ of streptomycin per ml. were found in 277 of the previously treated patients (20.4 per cent) (table 1).

The primary interest in the study was to determine the prevalence of streptomycin-resistant tubercle bacilli in patients who had never received streptomycin. There were 2,533 such patients in the study. Of these, 1,405 had cultures positive for tubercle bacilli. Drug-susceptibility determinations were reported for 1,175 of these, and the results are presented in table 2.

As may be seen in the table, an analysis of the 1,175 drug-susceptibility tests revealed that 42 patients (3.6 per cent) were discharging tubercle bacilli which were resistant to 10 γ of streptomycin per ml.

In order to obtain more information on these 42 persons who were reported as never having received streptomycin, a questionnaire was prepared requesting

[1] From the Research Service and the Reports and Statistics Service, Department of Medicine and Surgery, Veterans Administration Central Office, Washington, D. C.

[2] Presented at the Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, February 8–11, 1954, St. Louis, Missouri.

TABLE 1

BACTERIOLOGY: PATIENTS WITH PREVIOUS STREPTOMYCIN THERAPY

Total number of patients surveyed		2,993
Negative cultures		1,376
Positive cultures		1,617
Number not tested for drug sensitivity	257	
Number tested for drug sensitivity	1,360	
Susceptible	1,083	
Resistant	277	
Per cent resistant	20.4	

TABLE 2

BACTERIOLOGY: NO PREVIOUS STREPTOMYCIN THERAPY

Total number of patients surveyed		2,533
Negative cultures		1,128
Positive cultures		1,405
Number not tested for drug sensitivity	230	
Number tested for drug sensitivity	1,175	
Susceptible	1,133	
Resistant	42	
Per cent resistant	3.6	

TABLE 3

BACTERIOLOGY: NO PREVIOUS STREPTOMYCIN THERAPY
Based on Follow-up Information

Total number of patients surveyed		2,524
Negative cultures		1,128
Positive cultures		1,396
Number not tested for drug sensitivity	230	
Number tested for drug sensitivity	1,166	
Susceptible	1,136	
Resistant	30	
Per cent resistant	2.6	

confirmation of the drug-susceptibility determination as first reported, further details of the patient's previous tuberculosis contact history, and an evaluation of the response to subsequent chemotherapy.[1]

A review of these 42 cases revealed that 9 of the patients had received streptomycin before admission to the hospital; this reduced the number of cultures studied from patients with no previous streptomycin therapy to 1,166. In 3 instances, a laboratory error was found which decreased the number of patients who yielded streptomycin-resistant tubercle bacilli to 30 (2.6 per cent) (table 3).

A clear history of exposure to active cases of tuberculosis was established in 17 of these 30 patients who were discharging streptomycin-resistant tubercle

[1] Grateful acknowledgment is made to those persons who cooperated by reviewing these cases and submitting the additional data. The response to the questionnaire was 100 per cent.

TABLE 4

TUBERCULOSIS CONTACT HISTORY

Total number of patients	30
Hospital patients	11
Hospital employees	1
Family—social	5
Not established	13

TABLE 5

RESPONSE TO SUBSEQUENT TUBERCULOSIS CHEMOTHERAPY

Total number of cases	30
Improved	19
No change	3
Worse	1
Not reported	3
No streptomycin therapy	4

bacilli without any history of having received streptomycin. Eleven were exposed as patients in tuberculosis sanatoriums; one, as an employee; and 5, through familial contact. Nine of the 11 patients were hospitalized at tuberculosis hospitals after 1946, when streptomycin became available for clinical use. Of the remaining 2 patients, one was hospitalized in a tuberculosis sanatorium and the other in a general hospital, but the dates of their hospitalization are unknown. The employee worked on a tuberculosis ward in an Army hospital in 1945. Of the 5 patients with familial contact, one had contact in 1917 and the other 4 after 1946. Two patients with no definite history of contact were reported to be alcoholics, one of whom was further characterized as a "vagabond" (table 4).

Of the 30 patients with drug-resistant bacilli, 26 were subsequently treated with drugs. In 19, the tuberculosis responded favorably to streptomycin together with para-aminosalicylic acid (PAS) or isoniazid, and in one patient the disease responded to streptomycin alone. The favorable response of the tuberculosis in these patients was based upon clinical and roentgenographic improvement and disappearance of tubercle bacilli from sputum or gastric washings (table 5).

DISCUSSION

It is not surprising to find that 20.4 per cent of strains of *M. tuberculosis* isolated from tuberculous patients with a history of previous streptomycin therapy are resistant to 10 γ of streptomycin per ml., for prior to the time of this survey many patients received courses of streptomycin alone.

It is more difficult to explain the isolation of streptomycin-resistant tubercle bacilli from 2.6 per cent of the patients who had never received streptomycin. Although the present study does not represent a complete epidemiologic survey of these patients, the data suggest that some persons have been infected with streptomycin-resistant organisms through contact with tuberculous patients who may have received the drug. The history of contact with tuberculosis in this group is impressive, and most of the contacts occurred after streptomycin became

available for clinical use. It is therefore suggested from the data obtained in this study that exposure of patients in tuberculosis hospitals presents a likely source of infection with streptomycin-resistant tubercle bacilli.

The number of patients with streptomycin-resistant tubercle bacilli subsequently treated with chemotherapy is too small to draw any conclusions regarding the clinical significance of the finding of resistant bacilli. Furthermore, the clinical appraisal is further complicated by the fact that most of these patients who received subsequent chemotherapy were treated with multiple-drug regimens.

SUMMARY

Survey studies employing a "streptomycin-susceptibility test" revealed that 30 (2.6 per cent) of 1,166 patients admitted to Veterans Administration hospitals and not previously treated with streptomycin were found to yield tubercle bacilli resistant to 10 γ of streptomycin per ml. Many of these patients gave a clear history of contact with tuberculosis. On the basis of this study, it is suggested that infection of persons with streptomycin-resistant tubercle bacilli has not proved to be a serious public health hazard. Nevertheless, evidence is presented that infection of persons with streptomycin-resistant tubercle bacilli does occur.

SUMARIO

La Frecuencia de Bacilos Tuberculosos Estreptomicino-Resistentes en 5,526 Ingresos Consecutivos en el Hospital

Estudios exploradores que emplearon una "prueba de estreptomicino-sensibilidad" revelaron que 30 (2.6 por ciento) de 1,166 enfermos recibidos en los Hospitales de la Administración de Veteranos de los E. U. A. y no tratados antes con estreptomicina rendían bacilos tuberculosos resistentes a 10 γ de estreptomicina por ml. Muchos de esos enfermos comunicaron antecedentes indudables de contacto con tuberculosis. A base de este estudio, sugiérese que no está demostrado que la infección de personas con bacilos tuberculosos estreptomicino-resistentes constituya un riesgo sanitario grave. Preséntanse pruebas de que en verdad existe infección de personas por bacilos tuberculosos estreptomicino-resistentes.

RESUME

La p'édominance des bacilles tuberculeux streptomycine-résistants dans 5.526 cas consécutifs admis a l'hôpital

Des enquêtes utilisant le test de sensibilité à la streptomycine "streptomycin-sensitivity test" ont révélé que sur 1.166 malades admis dans les hôpitaux de la "Veterans Administration" qui n'avaient pas été soumis antérieurement au traitement par la streptomycine, 30 (2.6%) ont été trouvés porteurs de bacilles tuberculeux résistants à l'action d'une dose de streptomycine de 10 γ par ml. Chez un grand nombre de ces malades, il y avait des antécédents nets d'exposition à la tuberculose. Sur la base de ces travaux, les auteurs suggèrent qu'il n'est pas démontré que l'infection tuberculeuse des individus, par les bacilles streptomycine-résistants, constitue un danger sérieux en hygiène publique. Les auteurs présentent des données prouvant que l'infection d'individus par les bacilles tuberculeux streptomycine-résistants peut survenir.

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STUDIES ON ISONIAZID AND TUBERCLE BACILLI[1, 2]

II. The Growth Requirements, Catalase Activities, and Pathogenic Properties of Isoniazid-Resistant Mutants

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INTRODUCTION

It has previously been reported from this laboratory (1) and independently observed and reported by other investigators (2–7) that isoniazid-resistant, mutant strains of human and bovine tubercle bacilli are usually moderately or highly attenuated for guinea pigs. It has also been reported that such isoniazid-resistant strains manifest little or no catalase activity *in vitro* (8).

More recently it has been shown that at least three differentiable, isoniazid-resistant mutant types may be isolated from parent, wild-type, isoniazid-susceptible populations of tubercle bacilli under artificial conditions *in vitro* (9). In these same studies, it was observed that catalase-negative, isoniazid-resistant strains require, for the initiation of growth on agar, higher concentrations of hemin, alpha-keto acid, or, under certain conditions, inorganic iron than do isoniazid-susceptible, catalase-positive, fully pathogenic parent strains.

It will be seen that both types of isoniazid-resistant organisms may also appear in the discharges of patients treated with isoniazid, catalase-negative and catalase-positive (10).

The purpose of this communication is to describe in more detail observations on the differential growth requirements of catalase-positive and catalase-negative strains and to relate these observations to their different pathogenic properties.

MATERIALS AND METHODS

Strains: The stock laboratory strains of tubercle bacilli, H37Rv, Vallée, and Pearson, have previously been described; these parent, isoniazid-susceptible strains were identified as H37RvS, Vallée S, and Pearson S.[3] The isolation of catalase-negative, isoniazid-resistant (R^3) mutants of these strains has also been described (9). The resistant mutants of these stock laboratory strains were all capable of proliferating as well in the presence of 10 γ of isoniazid per ml. of medium as in the absence of isoniazid, provided the medium contained

[1] From the National Jewish Hospital at Denver, Colorado, and the University of Colorado School of Medicine.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[3] The suffix "S" applies in these studies to isoniazid-susceptible and "R" to isoniazid-resistant strains. During the course of these studies, the parent and isoniazid-resistant cultures of these strains were passed through animals (guinea pig or mouse) by infection and reisolated therefrom for the purpose of maintaining maximal virulence. The "Ry" mutant of the H37Rv strain, with properties previously described (9) was used in all experiments described here.

adequate amounts of bovine serum (fraction V) albumin (0.5 per cent), hemin, an alpha-keto acid, or catalase; they were routinely carried in the Tween®-albumin 7 H-5 medium described below.

The strains freshly isolated from pathologic materials were primarily cultured on Felsen plates containing the complete oleic-acid–albumin 7 H-3 solid agar medium described elsewhere (10) and subcultured in the Tween®-albumin 7 H-4 or 7 H-5 media described below. Their sources (from the control quadrant containing no drug, from the quadrant containing 1 γ of isoniazid per ml., or from the quadrant containing 10 γ of isoniazid per ml.) are specifically identified in the text under EXPERIMENTAL RESULTS.

All strains used in these studies were typical mammalian strain tubercle bacilli with regard to growth rate, microscopic morphology, microscopic and gross colony morphology, staining properties, and temperature requirements for optimal growth.

Culture media: For the routine maintenance of strains of tubercle bacilli in liquid media, the Tween®-albumin 7 H-4 medium was usually employed. This medium consisted of the basic medium (9) described previously, to which were added, before autoclaving, 0.005 gm. of ferric ammonium citrate per liter and 0.05 per cent Tween® 80. After autoclaving, 0.5 per cent albumin (0.1 volume of 5 per cent Seitz filter-sterilized solution of Armour fraction V dissolved in 0.85 per cent saline, adjusted to pH 6.8, and heated in a water bath at 56°C. for thirty minutes in 10 to 25 ml. tubes after incubation overnight at 37°C.) and 0.2 per cent glucose (reagent grade) from a 50 per cent solution in 0.005M citric acid, sterilized by autoclaving at 115°C. for fifteen minutes, were added.

To this medium was added either 0.1 γ or 10 γ of isoniazid per ml. before autoclaving for the routine maintenance of those strains having low (1 γ of isoniazid per ml.) or higher (>10 γ of isoniazid per ml.) resistance to isoniazid, as specifically referred to in the text. When 10 γ of isoniazid per ml. were added to the 7 H-4 medium, it was identified as medium 7 H-5.

All strains were routinely transferred weekly in these media by inoculation of 0.1 ml. of fully grown culture into 5.0 ml. of medium in Pyrex potato tubes (25 mm. O.D.) capped with aluminum hoods (Aloe-Willet).

The following special media were employed for the study of the special growth requirements of the various strains of tubercle bacilli for iron, iron compounds, alpha-keto acids, et cetera:

Special medium II: The following substances were dissolved in 1,000 ml. of distilled water: asparagine (Difco), 1.0 gm.; Na_2HPO_4 (anhydrous), 5.0 gm.; KH_2PO_4 (anhydrous), 2.0 gm.; $MgSO_4 \cdot 7H_2O$, 1.0 gm. The medium was adjusted to pH 7.5, and brought to a boil. It was allowed to cool to room temperature, which permitted flocculation of the insoluble magnesium compounds which adsorb heavy metals, including the traces of iron.

The medium was then filtered through Whatman paper, medium weight, which had been washed with 0.1 N hydrochloric acid and distilled water, to obtain a clear filtrate.

The iron-free filtrate was adjusted to pH 6.6.

The following salts were added in the final concentrations indicated: Na_3 citrate$\cdot 2H_2O$, 0.01 per cent; $CaCl_2 \cdot 2H_2O$, 0.00005 per cent; $CuSO_4 \cdot 5H_2O$, 0.0001 per cent; $ZnSO_4 \cdot 7H_2O$, 0.0001 per cent; and NH_4Cl, 0.02 per cent.

Serum albumin at 0.05 per cent and glucose at 0.5 per cent were added aseptically, after autoclaving.

Special medium III: This medium was identical with *special medium II* except that the L-glutamate (sodium salt) was substituted at 0.05 per cent final concentration for the asparagine.

The following vitamins were added after boiling and before autoclaving: biotin, 0.5 γ per ml. (final concentration); pyridoxin, 1.0 γ per ml. (final concentration). These vitamins were added from concentrated, sintered-glass-filter-sterilized solutions in distilled water.

Chemical reagents: Inorganic salts were all of reagent grade; manganous salts from two different sources were employed and were found to give identical results.

Bovine serum albumin obtained through the courtesy of the Armour Laboratories, Chicago, Illinois, as Cohn's fraction V of bovine serum was characterized as follows: It contained approximately 200 to 400 γ of total heme compounds per gm., as determined colorimetrically by quantitative benzidine test, and 20 to 40 γ of non-hemin iron, as determined colorimetrically with alpha, alpha-dipyridyl after reduction with sodium sulfite.

Vitamin preparations were obtained in crystalline form from Nutritional Biochemicals Corp., Cleveland 28, Ohio.

Hemin (crystalline) was obtained from two different sources, Nutritional Biochemicals Corp. and Eastman Kodak Co. They manifested identical properties in the experiments described here.

Organic acids, pyruvic and alpha-keto glutaric, lactic, L-glutamic, and oleic, were obtained from the Nutritional Biochemicals Corp. They were made up as neutral sodium salt solutions and used only after being freshly prepared. No significant differences were observed in the results described whether these acids were added before or after autoclaving to the media used. Only the alpha-keto acids reacted with hydrogen peroxide to yield carbon dioxide and water. The glutamic acid contained no demonstrable alpha-keto acid by this test.

Catalase was obtained as a dry powder from Nutritional Biochemicals Corp. under the label "Catalase (Lyophilized), Beef Liver, 40 to 50 milliequivalents of sodium perborate." It was dissolved in neutral, phosphate-buffered saline, sterilized by filtration through sintered glass, and stored at 4°C. in a concentration of 1 mg. per ml. It maintained its activity for periods of several months under these conditions without significant change. It was always added aseptically to the culture media in which it was used. One milliliter of a 1 mg. per ml. solution contained less than 10 γ of heme and no free iron detectable by alpha, alpha-dipyridyl test; and none of its activity in these studies, chemical or microbiologic, was dialyzable.

Glycerol was used as the reagent grade (Merck): C.P. and Technical grades were found to contain much more iron.

Glucose, as "Dextrose Anhydrous, Merck," was found to contain no significant amount of iron by either of two tests, alpha, alpha-dipyridyl or thiocyanate.

Inorganic iron source was ferric ammonium citrate, green scales, Merck (CP), containing 14.6 to 16 per cent iron by weight.

Conditions of incubation: All cultures were incubated for growth at 37° to 38°C., either in a large, walk-in incubator, exposed to atmospheric air or, when exposed to an atmosphere of 5 per cent carbon dioxide-air, in vacuum-desiccator jars, as indicated. The liquid medium cultures were shaken daily to permit good aeration, unless they were incubated in vacuum-desiccator jars.

Inocula: As routine, 5 ml. of test medium were inoculated with 0.05 ml. of fully grown culture from Tween®-albumin 7 H-4 medium. When well-dispersed cell suspensions were required, these were prepared, as previously described (9), by grinding and filtering Tween®-albumin or oleic-acid-albumin cultures; they contained approximately 2×10^8 bacterial units per 0.05 ml. as determined by plate count on appropriate media (with the use of separate pipettes for making each tenfold dilution). Ordinary, fully grown, Tween®-albumin cultures, not ground or filtered, contain approximately 5×10^6 bacterial units per 0.05 ml., many of these "units" consisting of clumps containing several bacterial cells.

Methods of infection and care of animals: Five different routes of infection were employed in the experiments described here: subcutaneous (1.0 ml. volumes in saline), intravenous in the marginal vein of the ear (1.0 ml. in saline), intracutaneous (0.1 ml. in saline), subcutaneous (1.0 ml. in saline), and air-borne (described below). Guinea pigs were maintained on a diet of "Sunco" (Monarch Mills) pellets, water in cups, and hay, *ad libitum*. Mice of the pure CF_1 strain (Carworth Farms, Inc.) were maintained on a diet of "rat and mouse" ("Monarch Lab-Menu", Monarch Mills) pellets and water from the usual inverted cups equipped with glass tubes from which the mice could lick, *ad libitum*. Rabbits were maintained on a diet of "Sunco" (Monarch Mills) pellets and water in cups, *ad libitum*.

All animals (except mice) were skin tested with 0.1 ml. of 1:10 or 1:100 dilution of Old Tuberculin and found to give no reactions before use in these studies.

Air-borne infection was performed in an air-borne infection apparatus by methods previously described (11), except that half-hour, instead of one-hour, exposures were used. The concentration of bacterial cells placed in the nebulizer for all of these experiments was 1:50 final dilution of a fully grown Tween®-albumin culture.

Special precautions were taken to ensure that the estimated numbers of bacterial cells (or units) employed for infection were actual. For all routes of infection, in all experiments, plate counts were made at 10^{-4} and 10^{-5} dilutions (0.1 ml. volume) of the undiluted suspensions on the 7 H-3 oleic-acid-albumin-agar medium (without the 0.2 per cent glycerin if Tween®-grown or Tween®-coated bacilli were used) in Petri dishes. In air-borne infection experiments, the additional precaution was taken to include at least five mice in each experiment, because, as will be seen, mice of the CF_1 strain are susceptible even to the catalase-negative, isoniazid-resistant strains of tubercle bacilli. When these mice are equally exposed, on the one hand, to isoniazid-susceptible, catalase-positive and, on the other, to isoniazid-resistant, catalase-negative strains, approximately equal numbers of grossly visible pulmonary lesions develop in their lungs by the twenty-eighth day after infection.

Tests for catalase activity: For determining the relative catalase activities of colonies of tubercle bacilli on solid agar media, the method previously described was applied (8); both the rate of appearance of bubbles and the total amount of bubbles formed during a period of approximately five minutes were taken into consideration in the roughly quantitative estimation permitted by this method. For determining in more quantitative fashion the relative amounts of catalase activity manifested by different strains of tubercle bacilli, a Warburg apparatus was used.[4] Cultures of equal optical density in the Tween-albumin 7 H-4 medium were directly introduced into the Warburg vessels and tested at 1°C. as will be described elsewhere in detail (12).

EXPERIMENTAL RESULTS

Studies on Growth Requirements

Requirements for inorganic iron or catalase of catalase-positive H37RvS and catalase-negative H37RvRy strains of tubercle bacilli in special medium II: Preliminary experiments with many types of artificial media led to the formulation of the liquid special medium II containing 0.05 per cent albumin for the study of the fastidious growth requirements of catalase-negative, isoniazid-resistant stock laboratory strains of tubercle bacilli. This medium contains no more than 0.02 γ of iron and 0.2 γ of hemin per ml. when special precautions are taken to wash all glassware used in its preparation and to use dilute hydrochloric acid (with low iron content) to rinse the culture tubes.

Experiments with large inocula of washed, ground and filtered, well-dispersed bacterial cells of the H37RvS and H37RvRy strains have shown that higher concentrations of inorganic iron, added to the special medium II, are required for the early initiation of growth of the H37RvRy strain than for the H37RvS strain.

In figure 1 it is shown that, with relatively small inocula (approximately 2×10^6 bacterial cells into 6 ml. of special medium II) of well-dispersed bacilli,

[4] The valuable cooperation and collaboration in these quantitative studies of Warren C. Morse and Robert Newmeyer at the Research and Development Laboratories, Fitzsimons Army Hospital, Denver, Colorado, are gratefully acknowledged.

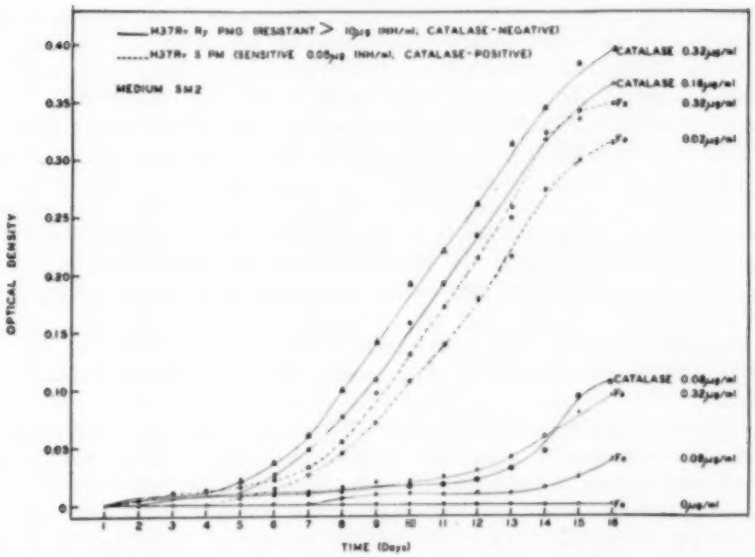

FIG. 1. Effects of inorganic iron and catalase on H37RvS and H37RvRy. Special medium II containing 0.02 per cent Tween® 80 with varying concentrations of catalase or inorganic iron, 6.0 ml. amounts in screw-cap culture tubes (Kimble Brand, 20 x 150 mm.), inoculated with 0.15 ml. washed, ground, filtered suspensions of fully grown seven-day cultures (Tween®-albumin 7 H-4 with 0.1 γ of catalase per ml.) of H37RvS and H37RvRy. Both suspensions were adjusted to 0.045 optical density in Beckman spectrophotometer, model DU, wavelength 480 mμ at 0.1 mm. slit opening. The tubes were incubated at 37C. in a planted position and shaken twice daily. Optical density readings were made at twenty-four-hour intervals, using Coleman, model 9, nepho-colorimeter, wavelength 525 mμ, with uninoculated special medium II as control.

the lag period for growth of the H37RvRy strain is markedly prolonged, compared with the H37RvS strain, even in medium containing added inorganic iron (0.32 γ of iron per ml.).

It is also recorded in figure 1 that the catalase-negative strain multiplies as well as the catalase-positive strain in special medium II containing added catalase (0.16 γ per ml.), even without added inorganic iron.

The possibility that phenotypic or genotypic change to catalase-positivity is responsible for the growth which usually occurs after a long lag period in minimal-iron medium inoculated with the H37RvRy organisms has been eliminated in several separate experiments.

It should be emphasized that the results with this type of experiment have been moderately irregular, depending upon the particular batch of medium and the size and source of the inoculum. Well-dispersed suspensions of bacterial cells gave the clearest results.

Differential toxicity of manganese for catalase-positive and catalase-negative

strains: Early in the course of these studies it was observed that manganous ions in trace amounts were selectively inhibitory for the growth of the catalase-negative H37RvR (*x* and *y*) (9) and H37RvRy strains. In many separate experiments during the course of these studies it has been shown that, in the special medium II, manganese at a final concentration of 0.005 γ per ml. is inhibitory for the H37RvRy strain, whereas as high a concentration as 25 γ of manganese per ml. has no inhibitory effect under the same conditions for the H37RvS strain. Thus, in this medium, manganese is more than five thousand times as toxic for the H37RvRy as for the H37RvS strain. This activity of manganese is not shared by any other metal thus far examined, nickel, cobalt, cuprum, zinc, calcium, and magnesium, and is not antagonized by these other metals.

Essentially the same results have been obtained in identical studies with the Vallée and Pearson strains and their catalase-negative, isoniazid-resistant mutants.

Antagonism of manganese toxicity by inorganic iron, hemin, pyruvate, catalase, and serum albumin: In many experiments, too extensive to permit detailed description herein, it has repeatedly been observed that only inorganic iron, hemin, alpha-keto acids, purified catalase, and serum albumin have been reproducibly antagonistic to the toxic effect of manganese on the growth of catalase-negative, isoniazid-resistant mutants of the H37Rv, Vallée, and Pearson strains. Only these substances, among a wide variety of well-characterized organic and inorganic materials examined, have shared this property.

In table 1 are shown the minimal requirements of some of these substances for growth of the H37RvRy strain in the special medium II in the presence of added manganese (0.02 γ per ml.).

The results of an experiment which shows the effect of size of inoculum on

TABLE 1

ANTAGONISM OF MANGANESE TOXICITY FOR H37RvRy BY INORGANIC IRON, HEMIN, PYRUVIC ACID

Iron	Incubation in Days		Hemin	Incubation in Days		Pyruvate	Incubation in Days	
	7	14		7	14		7	14
γ *per ml.*			γ *per ml.*			γ *per ml.*		
4.8	++	+++	6.4	+++	++++	40.0	+++	++++
2.4	+	+++	3.2	++	++++	20.0	+++	++++
1.2	+	+++	1.6	+	+++	10.0	+++	+++
0.6	+	+++	0.8	−	±	5.0	++	+++
0.3	±	+	0.4	−	−	2.5	±	±
0.15	−	±	0.2	−	−	1.25	−	−
0	−	−	0	−	−	0	−	−
0.8 Mn^{++}	+++	++++	0.8 Mn^{++}	+++	++++	0.8 Mn^{++}	++++	++++

Special medium II with final concentration of 0.02 γ of manganese per ml.

Inoculum, 0.05 ml. of fully grown Tween®-albumin 7 H-4 culture of H37RvRy per 5.0 ml. of medium.

Amount of growth expressed as none (−) to maximal (++++).

TABLE 2

EFFECT OF SIZE OF INOCULUM ON INHIBITION OF H37RvRy BY MANGANESE

Ml. of Inoculum into 5.0 Ml. Medium	γ of Manganese per Ml.						
	0	0.0012	0.0025	0.005	0.01	0.02	0.04
0.05	++	++	+	±	−	−	−
0.1	+++	+++	+++	++	+	±	−
0.2	++++	+++	+++	+++	+++	±	−

Special medium II.

Varying amounts of inoculum from fully grown Tween®-albumin 7 H-4 culture of H37RvRy.

Readings after seven days' incubation.

Amount of growth expressed as none (−) to maximal (++++).

TABLE 3

ANTAGONISM OF MANGANESE TOXICITY BY CATALASE

γ of Manganese per Ml.	γ of Catalase per Ml.									
	0	0.003	0.06	0.012	0.025	0.05	0.1	0.2	0.4	$\frac{0.5}{Mn^{++}}$
0.02	−	−	±	++	++	+++	+++	+++	+++	++
0.2	−	−	−	±	++	+++	+++	+++	+++	++
2.0	−	−	−	−	±	+	+++	+++	+++	++

Special medium II.

Inoculum, 0.05 ml. of fully grown Tween®-albumin 7 H-4 culture of H37RvRy per 5.0 ml. of medium.

Readings after seven days' incubation.

Amount of growth expressed as none (−) to maximal (++++).

the inhibition of growth of the H37RvRy strain by manganese are shown in table 2.

The results of an experiment which illustrates the quantitative effects of various concentrations of manganese on the requirement of the H37RvRy strain for catalase are described in table 3.

Alpha-keto glutaric acid is, molecule for molecule, as effective as pyruvic acid in its role as a growth factor for the catalase-negative, isoniazid-resistant mutants of the H37Rv, Vallée, and Pearson strains under these conditions *in vitro*.

Lactic acid does not antagonize the manganese toxicity for catalase-negative strains, nor does it decrease the activity of alpha-keto acids as manganese antagonists.

Effect of isoniazid on the special growth requirements of H37RvRy: The presence of isoniazid in either the inoculum or the special medium II is not necessary for the manifestations of the special requirements of catalase-negative, isoniazid-resistant strains of tubercle bacilli. However, the presence of isoniazid in the medium increases somewhat these special growth requirements of such

TABLE 4

EFFECT OF ISONIAZID ON THE ANTAGONISM OF MANGANESE TOXICITY FOR H37RvRy BY HEMIN

γ of Hemin per Ml.	0.02 γ of Mn^{++} per Ml. of Special Medium II		0.02 γ of Mn^{++}, 5.0 γ of Isoniazid per Ml. of Special Medium II	
	Incubation in Days		Incubation in Days	
	7	14	7	14
0	−	−	−	−
0.5	−	−	−	−
1.0	−	±	−	−
2.0	+	+++	−	−
4.0	++	++++	−	±
8.0	+++	++++	++	++++
16.0	+++	++++	+++	++++

Inoculum, 0.05 ml. of fully grown Tween®-albumin 7 H-4 culture of H37RvRy per 5.0 ml. of medium.

Amount of growth expressed as none (−) to maximal (++++).

TABLE 5

ANTIBACTERIAL ACTIVITY OF HYDROGEN PEROXIDE FOR TUBERCLE BACILLI

	Minimal Bacteriostatic* Concentration of Hydrogen Dioxide
	γ *per ml.*
H37Rv-Isoniazid-susceptible	10
H37Rv-Isoniazid-resistant	0.8
Vallée-Isoniazid-susceptible	10
Vallée-Isoniazid-resistant	0.5

* These same concentrations sterilize more than 99 per cent of the bacterial cells within twenty-four hours at 38°C.

Medium: oleic-acid–albumin liquid medium (7 H-4) containing no alpha-keto acid.

strains. The results of an experiment which illustrates this phenomenon are shown in table 4.

Toxicity of hydrogen peroxide for catalase-negative and catalase-positive strains: In view of the apparent deficiency of certain isoniazid-resistant strains of tubercle bacilli in "catalase activity," experiments were performed to determine whether or not the catalase-deficient strains are more susceptible than the catalase-positive strains to the toxic effects of hydrogen peroxide *in vitro*. That this is true is shown in table 5, in which an experiment is described with two parent, isoniazid-susceptible strains and their catalase-negative, isoniazid-resistant mutants. The high susceptibility of the catalase-negative strains to hydrogen peroxide, in both bacteriostatic and bactericidal tests, is obviously consistent with their deficiency in catalase activity.

Bacteriostatic versus bactericidal activity of manganese on catalase-negative strains: A few experiments have been performed with the aim of determining

whether the selective inhibition of manganese for the catalase-negative H37-RvRy strain is limited to a bacteriostatic effect or is also bactericidal. These experiments have given unexpected and thus far unexplainable results as follows:

1. The addition, within forty-eight hours, of an antagonist of manganese toxicity (catalase or pyruvate) in excess of the amount required for antagonism of bacteriostasis by manganese usually results in the resumption of good growth of the inoculum.

2. Addition of the same amounts of manganese-antagonists at a later time (up to fourteen days) after inoculation and incubation at 37° to 38°C. results within a few days in a slight amount of subsequent bacterial multiplication which is strictly limited; even after two more weeks of incubation, no further growth is observed. Stained smears of the bacilli during the course of these experiments reveal that they do not lose their acid-fastness or change in other morphologic respects.

Thus far, all such experiments have been carried out in liquid media, without quantitative plate counts of selected samples and under ordinary atmospheric air.

The results of these experiments suggest that manganese exerts a subtle and, as yet, little understood, sterilizing effect, in addition to an obvious bacteriostatic effect, on this catalase-negative, isoniazid-resistant strain at 37°C. in the special growth medium II.

Growth requirements of additional strains of tubercle bacilli: Because of the results obtained with the stock, laboratory, H37Rv, Vallée, and Pearson strains, several other strains of tubercle bacilli have been studied: catalase-positive, isoniazid-susceptible strains; catalase-positive (weakly or moderately positive) strains resistant to at least 1 γ of isoniazid per ml. *in vitro*; and catalase-negative strains, resistant to 10 γ of isoniazid on primary isolation from pathologic materials. In table 6 are recorded the results of this study.

These strains were recently isolated from human pathologic materials in this laboratory. The isoniazid-susceptible strains were derived by one subculture in the Tween®-albumin 7 H-4 liquid medium from specimens yielding extensive growth on the control quadrant of a Felsen plate containing oleic-acid–albumin 7 H-3 medium (to be described in a separate communication (10)). This isoniazid-susceptible strain yielded no growth on the quadrants containing 1 γ or 10 γ of isoniazid per ml.; the isoniazid-resistant strains were isolated from the 10 γ isoniazid quadrant of similar primary isolate cultures, and were, therefore, pure cultures of tubercle bacilli resistant to at least 10 γ of isoniazid per ml. of this medium. These strains were subcultured only once in the Tween® albumin 7 H-5 medium (containing 10 γ of isoniazid per ml.).

The special medium III, as well as the special medium II, was employed because of the observation that some strains of tubercle bacilli may be inhibited by asparagine and others may require the added vitamins, biotin or pyridoxin, for optimal growth (10).

In general, the results show that, on the one hand, all the catalase-positive strains, including those which are resistant to 1 γ or more of isoniazid per ml., are capable of growing as well in the special medium II, or special medium III, with added manganese as in the same medium with added catalase. On the

TABLE 6

CATALASE ACTIVITY AND SUSCEPTIBILITY TO MANGANESE TOXICITY OF STRAINS ISOLATED FROM PATIENTS' SPUTA

Description of Strain	Number of Strains Studied	Special Medium II			Special Medium III		
		0	0.2 γ of Mn^{++} per Ml.	0.2 γ of Mn^{++} per Ml., 1.0 γ of Catalase per Ml.	0	0.2 γ of Mn^{++} per Ml.	0.2 γ of Mn^{++} per Ml., 1.0 γ of Catalase per Ml.
Isoniazid-susceptible, *catalase ++ to +++	5	+++	+++	+++	+++	+++	+++
Isoniazid-resistant catalase + to ++	4	+++	+++	+++	+++	+++	++++
Isoniazid-resistant catalase −	14	+	−	+++	++	−	+++
	6	+++	±	+++	+++	±	++++
	1†	+++	+++	+++	+++	+++	+++

* Test for catalase activity performed on oleic-acid-albumin 7 H-3 agar medium (and in some cases, in Warburg apparatus).

† A highly attenuated strain (MK No. 10), resistant to >10 γ of isoniazid per ml. (*see* text).

Amount of growth expressed as none (−) to maximal (++++).

Readings after seven days' incubation.

other hand, the strains which manifest no catalase activity *in vitro* are inhibited by manganese (0.2 γ per ml.) under these conditions, and this inhibition by manganese is antagonized by added catalase.

The one strain (MK No. 10) in this series which was not inhibited under these conditions by 0.2 γ of manganese per ml., although it is catalase-negative,[5] was for this reason the subject of special study in further experiments. It was shown to be highly attenuated for guinea pigs (*see* below). Therefore, it was postulated that, under appropriate conditions *in vitro*, it should manifest susceptibility to manganese. The possibility was entertained that this strain differed from the other catalase-negative strains encountered in this series by virtue of a peculiarly active system for oxidatively deaminating asparagine or glutamic acid to their alpha-keto derivatives. This might permit antagonism of the toxicity of manganese without the aid of an exogenous source of catalase or catalase-like substances. This hypothesis was tested. However, even in the absence of added catalase, the MK No. 10 strain was not inhibited by 0.2 γ of manganese per ml. Thus, this strain, perhaps unique in this respect, deserves further special study.

It should be pointed out that, although the catalase-positive, resistant strains

[5] There is a possibility that this strain has very weak but definite catalase activity. Quantitatively sensitive Warburg studies have not yet been carried out with this strain.

described here (except for the MK No. 1 strain) were resistant to 10 γ as well as to 1 γ of isoniazid per ml. of oleic-acid–albumin 7 H-3 medium, the great majority of the 20 catalase-positive, isoniazid-resistant strains found in primary isolate cultures from pathologic materials were resistant to 1 γ, but susceptible to 10 γ, of isoniazid per ml. (to be described in a separate communication (10)). The results of catalase tests were consistent, therefore, with the results of guinea pig virulence tests previously described in these laboratories (1) and by Mitchison (5). The majority of only moderately attenuated isoniazid-resistant strains isolated from clinical material are resistant to 1 γ, but susceptible to 10 γ, of isoniazid per ml.

Pathogenicity Studies

Pathogenic properties of the H37RvS and of the H37RvRy strains: In several separate experiments, the pathogenicities of parent, catalase-positive, and catalase-negative, isoniazid-resistant ($>10\ \gamma$ of isoniazid per ml.) mutants of the H37Rv, Vallée, and Pearson strains have been compared. This was done by challenging normal guinea pigs by the intravenous, air-borne, and subcutaneous routes and mice by the air-borne infection route. The results of these experiments have been essentially uniform. In the dosages used, the catalase-positive, parent strains have manifested pathogenic properties consistent with those considered typical of fully virulent mammalian strain tubercle bacilli. In contrast, the catalase-negative mutants have invariably shown themselves to be highly attenuated for guinea pigs but fully pathogenic for mice under the same experimental conditions.

Suffice it to describe here in some detail the results of a single study in which the H37RvS and the H37RvRy strains were compared.

Well-dispersed (ground and filtered) suspensions of these two strains were prepared as previously described. On being plated out, these suspensions yielded 69 colonies for the parent, and 51 for the resistant, strain at 0.1 ml. of 10^{-5} dilution. Thus, they each contained approximately 5×10^7 bacilli per ml. Six groups of 6 guinea pigs each were challenged in this experiment: one group, with each strain, by air-borne infection; one group, by the intravenous route (1.0 ml. of 10^{-2} dilution, or approximately 5×10^5 bacilli); and one group, by the subcutaneous route in the groin (1.0 ml. of 10^{-2} dilution).

Tuberculin (OT) skin tests were performed on every animal before infection and at two, three, and four weeks after infection. Only those guinea pigs infected by the subcutaneous route manifested a hypersensitive reaction to 1:100 OT at two weeks; and only the animals in this same group gave moderate to marked reactions to this dilution of OT at three and four weeks after challenge.

Three animals in each group either died before or were sacrificed at thirty days after infection. The accompanying illustrations (figures 2 and 3) show the gross appearance of the lungs and spleens of 2, unselected, guinea pigs from each of the groups infected by the *air-borne route* and of the lungs of 4 mice simultaneously exposed to these same two strains in this experiment. Only a few isolated, grossly visible lesions were observed on the lungs of the guinea

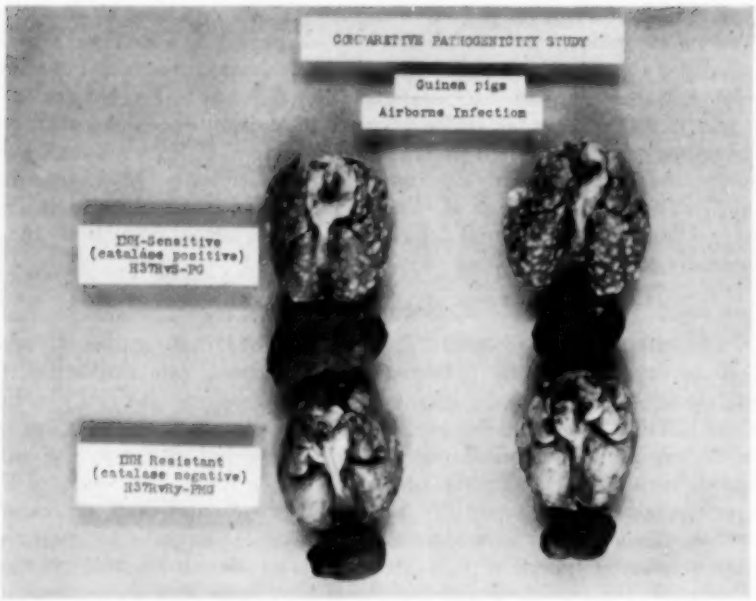

FIG. 2. Gross appearance of the formol-fixed lungs and spleens of guinea pigs infected (twenty-eight days) with equal numbers of isoniazid-susceptible, catalase-positive (above) and isoniazid-resistant, catalase-negative (below) tubercle bacilli from the H37Rv strain. Note the comparatively small spleens and rare, small lesions on lungs of animals challenged with the catalase-negative bacilli.

pigs infected with the H37RvRy strain, whereas the guinea pigs challenged with the H37RvS strain had multiple large lesions. Indeed, 3 of the guinea pigs challenged with the H37RvS strain had died with similarly extensive pulmonary lesions at twenty-six, twenty-seven, and twenty-seven days, respectively, after infection.

The results of this experiment did not reveal any differences between the drug-susceptible and drug-resistant strains in mice—an observation of some significance in view of the fact that pulmonary lesions can be induced only by a single bacillus or by clumps containing very few bacilli. Nor did histologic sections of the mouse lesions reveal any significant differences in the lesions, either with respect to the histologic response or the numbers of acid-fast bacilli, which were very numerous. On the other hand, stained histologic sections of the lungs of the guinea pigs revealed marked differences, quite consistent with their gross appearances. No acid-fast rods were seen in Ziehl-Neelsen stained sections of the grossly observed lesions in the lungs of guinea pigs challenged with the drug-resistant strain, and such lesions were "healing" in the sense that fibroblastic proliferation and round-cell infiltration were marked at their periphery.

FIG. 3. Gross appearance of the formol-fixed lungs of CF_1 mice infected (twenty-eight days) with equal numbers of isoniazid-susceptible, catalase-positive (above) and isoniazid-resistant, catalase-negative (below) tubercle bacilli from the H37Rv strain. Note the equal numbers of lesions and identical appearance (confirmed histologically), compared with the results in guinea pigs simultaneously infected (figure 2).

Four guinea pigs infected with the drug-susceptible strain by the *intravenous route* died at twenty-four, thirty-three, thirty-three, and fifty-seven days; 2 were sacrificed on the thirtieth day. All had grossly enlarged, necrotic spleens, positive for acid-fast rods on direct smear and yielding many colonies of tubercle bacilli at 10^{-3} dilution of ground suspensions. Numerous tubercles, from pinpoint size to larger, were found on the lungs of 5 of these 6 animals.

Three of the guinea pigs infected with the drug-resistant strain were sacrificed at thirty days. No lesions were grossly visible on their lungs, but their spleens were slightly enlarged and resistant organisms were readily isolated therefrom. Two of them died of extraneous cause on the forty-fourth and sixty-sixth days, respectively, showing no gross evidence of tuberculous disease. The one surviving animal was killed on the eighty-fifth day; it showed no anatomic evidence of having been challenged with living tubercle bacilli.

Three of the 6 animals which had been challenged by the *subcutaneous route* with the drug-susceptible strain were sacrificed at thirty days after infection. Two of these already manifested grossly diseased spleens as well as enlarged local, draining nodes. One was sacrificed by accident at thirty-nine days; it already had a few pulmonary tubercles in addition to an enlarged tuberculous

spleen and a local abscess at the site of inoculation. Another died of massive pulmonary tuberculosis at seventy-five days. The animal which survived and was sacrificed at eighty-five days also had typical visceral tuberculous disease.

The 3 guinea pigs which had been challenged with the drug-resistant strain by the subcutaneous route and were sacrificed on the thirtieth day had small local abscesses at the sites of injection and small local nodes. No visceral disease was evident in spite of the fact that a few catalase-negative, isoniazid-resistant colonies appeared on medium inoculated with an undiluted, ground spleen suspension from one of these animals. The remaining 3 animals were sacrificed at eighty-five days. Only walled-off, local abscesses were found at the sites of challenge in these animals.

In certain air-borne infection experiments the H37RvRy strain has manifested greater "infectivity," although no more "virulence" in terms of its ability to produce continuously progressive disease, than was shown in this experiment. In particular, it can be seen in figure 4 that, under certain, as yet ill-defined, conditions, more lesions may be produced within twenty-eight days after infection by the air-borne route. Such lesions showed histologic evidences of healing by the twenty-eighth day after challenge, and they contained very few acid-fast rods, compared with lesions in guinea pigs similarly examined twenty-eight days after air-borne challenge with the H37RvS strain.

Pathogenicity of Vallée S and Vallée R for immunized rabbits: The use of the intracutaneous route for challenge has been exploited in studies on the comparative pathogenicities of the catalase-positive, isoniazid-susceptible, bovine Vallée strain and its catalase-negative mutant in rabbits. Infection of 8 immunized rabbits[6] by this route simultaneously with 0.1 ml. of 10^{-3} dilutions of Tween®-albumin cultures of both these strains revealed that until the fourteenth day the local lesions were similar in their development; after this period, the Vallée S strain produced ulcers which persisted until the animals were sacrificed, while the local nodules which developed at the sites of injection of the Vallée R strain gradually disappeared entirely.

Special studies on a catalase-positive, isoniazid-resistant strain, and a catalase-negative, isoniazid-resistant strain from the same sputum specimen: A specimen of sputum from M.K., No. 11434, was submitted to the clinical laboratory for routine bacteriologic studies for tubercle bacilli. The results of primary culture, drug-resistance, and catalase tests (*see* subsequent communication, (10)) showed that it was a "special mixed" specimen. Both catalase-negative organisms, resistant to 10 γ of isoniazid per ml., and catalase-positive organisms, resistant to at least 1 γ of isoniazid per ml., were present in the specimen. A few colonies were selected from the quadrant containing 10 γ of isoniazid per ml. and subcultured in Tween®-albumin 7 H-5 medium; a few from the quadrant containing 1 γ of isoniazid per ml. were similarly subcultured in Tween®-albumin 7 H-4

[6] Two immunized by previous infection with very small doses of a virulent bovine (Vallée) strain of tubercle bacilli; 2, with large doses of a virulent human (H37Rv) strain; 4, injected with heat-killed tubercle bacilli (H37Rv), 1 mg. dry weight, by the intravenous route.

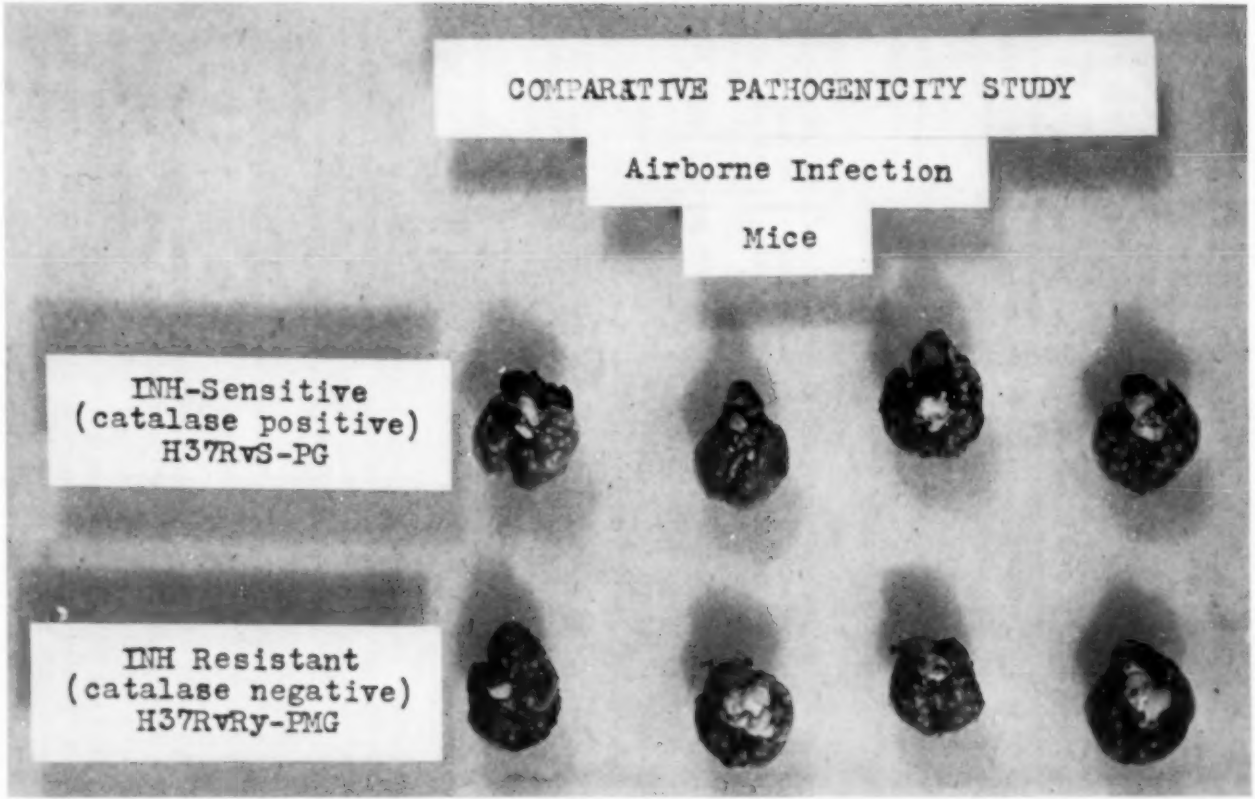

FIG. 4. Freshly removed lungs of guinea pigs challenged twenty-eight days previously by the air-borne route with isoniazid-resistant, catalase-negative bacilli from the H37Rv strain. Compare with results obtained in a similar experiment with similar culture (figure 2). Histologic examination of lesions revealed very few acid-fast rods and evidences of "healing.

TABLE 7

COMPARATIVE PATHOGENICITIES OF TWO ISONIAZID-RESISTANT STRAINS, AND THE RESPONSE OF GUINEA PIGS TO THERAPY WITH ISONIAZID

Intravenous Infection with	Treatment	Necropsies (Twenty-Eighth Day)		Culture Recoveries* 0.1 Ml. of 10^{-1} Dilution of Lung Suspensions
		Gross	Microscopic (Lungs)	
Mixed culture MK No. 1 (catalase +++) 1×10^7 bacilli MK No. 10 (catalase −) 4×10^7 bacilli	1 ml. of saline 2 times daily (6 animals)	(1 death; twenty-seventh day) Extensive miliary tuberculosis Average weight of spleens: 3.6 gm.	Many necrotic foci; numerous a.f.b.†	>300 colonies. Catalase activities: >20 catalase-positive; 1 catalase-negative
	5 mg. of isoniazid per kg. 2 times daily (6 animals)	(2 deaths: 20, 24 days) Extensive miliary tuberculosis Average weight of spleens: 2.8 gm.	Many necrotic foci; numerous a.f.b.	171 colonies; 142 colonies. Catalase activities: >20 catalase-positive; 1 catalase-negative
	20 mg. of isoniazid per kg. 2 times daily (6 animals)	Fine tubercles on lungs Average weight of spleens: 1.6 gm.	Many necrotic foci (round-cell infiltration); rare a.f.b.	24 colonies; 25 colonies. Catalase activities: c. 20 catalase-positive; 1 catalase-negative
Pure culture: MK No. 10 (catalase−) 5×10^7 bacilli	None (6 animals)	Fine tubercles on lungs Average weight of spleens: 1.8 gm.	Many non-necrotic foci (round-cell infiltration); rare a.f.b.	15 colonies; 10 colonies. Catalase activities: all catalase-negative

* On "complete" oleic-acid-albumin 7 H-3 agar medium; duplicate plates.
† a.f.b.—acid-fast bacilli.

medium (plus 1 γ of isoniazid per ml.). The first culture was labeled M.K. No. 10, and the second M.K. No. 1. These two cultures were permitted to attain full growth and were then used (not specially dispersed) to infect guinea pigs by the intravenous route. Three groups of 6 guinea pigs each were injected with 1.0 ml. of 10^{-1} dilution of the M.K. No. 1 culture and one group of 6 with the same dose of the M.K. No. 10 culture. The guinea pigs of one of the three groups

challenged with the M.K. No. 1 culture and of the one group challenged with the M.K. No. 10 strain were injected twice daily (at 9 a.m. and at 5 p.m.) with 1.0 ml. of saline, beginning on the day of infection. The animals in the second group challenged with the M.K. No. 1 culture were injected with 5 mg. of isoniazid per kg. twice daily; those of the third group, with 20 mg. of isoniazid per kg. twice daily.

The results of this experiment are recorded in table 7. It will be noted that the M.K. No. 1 culture contained both catalase-negative and catalase-positive organisms in the proportion of approximately 4:1, whereas the M.K. No. 10 culture proved to be a "pure" strain of catalase-negative organisms resistant to 10 γ of isoniazid per ml. Moreover, the catalase-positive organisms in the M.K. No. 1 culture were in subsequent studies shown to be resistant to 3 γ, but susceptible to 10 γ, of isoniazid per ml. of oleic-acid–albumin 7 H-3 medium.

The results of these experiments clearly show that the catalase-positive, isoniazid-resistant mutant is much more pathogenic for guinea pigs than is the catalase-negative mutant strain. The results further show that large doses of isoniazid are chemotherapeutically active in suppressing disease due to the catalase-positive mutant which is resistant to 3 γ, but susceptible to 10 γ, of isoniazid per ml. Histologic sections stained for acid-fast rods have confirmed the results observed macroscopically, which have been described above. Very few acid-fast rods were seen in lesions of the animals challenged with the M.K. No. 1 mixed culture and treated with 40 mg. of isoniazid per kg. per day or in those challenged with the pure strain M.K. No. 10 culture, compared with the other animals.

It is important to add that one additional catalase-positive strain resistant to 1 γ, but susceptible to 10 γ, of isoniazid per ml., and three strains (two previously reported (8)) catalase-positive and resistant to 10 γ as well as to 1 γ of isoniazid per ml. have been found to be only moderately attenuated for guinea pigs. These four strains multiplied and caused fatal tuberculosis after intravenous injection and, in some guinea pigs, produced visceral tuberculous lesions after challenge with large numbers of living bacilli by the subcutaneous route (1 ml. of 10^{-2} dilution of Tween®-albumin 7 H-4 cultures). Isoniazid-resistant, catalase-positive bacilli were isolated from the visceral lesions.

Clinical laboratory studies of pathogenicity of tubercle bacilli in sputum concentrates: In confirmation and extension of the previous report on the pathogenicity of tubercle bacilli in 21 specimens from 21 different tuberculous patients treated with isoniazid (1), 28 additional specimens from 28 different patients have been studied. Twenty-five of these patients had been treated with isoniazid, 3 to 5 mg. per kg. per day for periods from four days to twelve months; 3 had not been treated with isoniazid. All 28 of these patients had advanced pulmonary tuberculosis, and smears of all specimens were positive for acid-fast rods after concentration (2/20 to 40/1 oil-immersion field). Each of two guinea pigs were challenged in the groin with the concentrated material from each specimen.

The following results were obtained:

Only 10 of these specimens yielded strains which were significantly pathogenic for guinea pigs (capable of multiplying to produce grossly visible visceral lesions within sixty-one days). Positive cultures were obtained from the diseased spleens of all of the guinea pigs challenged with these specimens.

In 7 of these instances, all of the organisms (total population) recovered from the diseased spleens were inhibited by 1 γ of isoniazid per ml. The 3 specimens from patients not treated with isoniazid were in this group.

In 3 instances, the total population isolated was apparently resistant to 1 γ of isoniazid per ml. but susceptible to 25 γ per ml. Of special importance are the subsequent observations that these 3 strains, which were at least moderately pathogenic for the guinea pig, were catalase-positive strains, although they were resistant to 1 γ of isoniazid per ml. of medium.

The tubercle bacilli in the remaining 18 specimens did not show the usual pathogenicity for guinea pigs, and no bacteriologic recoveries were obtained from the guinea pig spleens, all of which were submitted to culture.

DISCUSSION

The special growth studies described in this paper confirm and extend the observations previously described (9) for the growth of isoniazid-resistant tubercle bacilli on direct isolation from parent, isoniazid-susceptible strains *in vitro*. It is seen that all of the chemical agents which strikingly promote the growth of the catalase-deficient strains of drug-resistant organisms are substances which have catalase or catalase-like activity—catalase, hemin, alpha-keto acids.[7]

The role of inorganic iron as a growth-supportive for such strains is not entirely clear. It seems possible that concentrations of inorganic iron in excess of the minimal concentration required for good growth of the catalase-producing strains may favor more efficient operation of their cytochrome system. It is generally assumed that the complete cytochrome system operates in terminal respiratory metabolism by direct transfer of hydrogen to molecular oxygen without the production of hydrogen peroxide. A second possibility, nevertheless, is that small amounts of certain iron oxides, which have been shown to have catalase activity (13, 14), are slowly formed in the incubated medium from the added Fe^{+++}. In any event, the growth-promoting activity of inorganic iron is slight in the present experiments, compared with those substances having marked "catalase" activity.

Serum albumin, as it is obtained commercially, manifests catalase activity of a low order of magnitude in Warburg studies (12); this activity may be attributed to its content of hemin or to actual contamination with catalase. Thus, the growth-promoting activity of serum albumin in these studies may also be attributed to its "catalase-activity" as well as to its content of inorganic iron.

[7] It is well known that alpha-keto acids react spontaneously and nonenzymatically with hydrogen peroxide to decompose it to water and be themselves decomposed to the next lower carbon acid plus carbon peroxide.

The remarkable activity of manganese in increasing the metabolic embarrassment of catalase-deficient strains in catalase-free medium is noteworthy. Several recent studies with different bacterial species suggest that manganese may be an essential metal in bacterial metabolism (15, 16). Indeed, it seems probable that the special medium II, as prepared in this laboratory, is not free of traces of manganese. This would account for the failure of small, well-dispersed inocula of catalase-negative strains to thrive in the basic medium even without the addition of manganese. Unfortunately, the present writers know of no method which permits the detection of the minute traces of manganese ($<0.005\ \gamma$ of manganese per ml. of liquid medium or per 5 mg. of ashed medium) which would account for the results obtained in the basic medium without added manganese.

The concentration of "free" manganese in animal tissues, not to mention its concentration in the biochemical environment in which tubercle bacilli multiply *in vivo*, is not established. However, the recent studies of Fore and Morton (17) on manganese in rabbit tissues seem to justify the present practice of adding at least $0.2\ \gamma$ of manganese per ml. of medium for clearly revealing the requirements of catalase-negative strains for catalase or agents having similar organic peroxide- or hydrogen peroxide-disposing activity.

None of the present studies have shed any light on how manganese, so specific in its toxic action on all but one of several catalase-negative strains, exerts its obviously important biochemical effects.

The report of Fisher (18), confirmed by Barry, Conalty, and Gaffney (3) and by the present writers, in which the type of medium employed by Fisher was used, led to the suggestion (1) that "most strains of tubercle bacilli which are resistant to more than $10\ \gamma$ of isoniazid per ml. of artificial medium have a special growth requirement for a substance (or substances) which, on the one hand, is present but bound and unavailable in normal tissue, but, on the other hand, free and available to these fastidious strains in necrotic tissue."

Fisher (19*a* and *b*) has subsequently presented evidence that hemin can function as a growth factor for certain isoniazid-resistant strains of tubercle bacilli. This observation was interpreted as indicating "that the loss of a hemin-synthesizing mechanism renders tubercle bacilli resistant to inhibition of growth by isoniazid." However, a catalase-negative, isoniazid-resistant strain of tubercle bacillus, cultivated in this laboratory in a hemin-deficient medium containing inorganic iron adequate for growth, has been shown to synthesize as much heme as the catalase-positive, isoniazid-susceptible parent strain from which it was derived (20). For this, as well as for other reasons, hemin cannot be regarded in any strict sense as a unique growth factor for any of the strains of tubercle bacilli encountered by the present writers.

The experimental data presented in this report permit the general conclusion that isoniazid-resistant strains of tubercle bacilli which are highly attenuated for guinea pigs manifest only an apparent, not a real, nutritional deficiency. Their special growth requirements are a consequence of their metabolic deficiency in catalase activity and under no conditions of growth thus far encountered *in vitro* is their catalase-activity restored. Indeed, it seems fair to

assume that, for these organisms, beef liver catalase, with its molecular weight of 248,000 (21), acts as a "growth-protective" or "growth-supportive" factor at or very near the surface of each bacillus. It appears unlikely that it acts as a nutritional growth factor in the strictest sense—penetration through the bacterial cell wall and intracellular utilization. Furthermore, it should be pointed out that the catalase deficiency of such strains may be the result either of a failure to synthesize catalase or of the presence of an inhibitor of the preformed enzyme.

The fact that not all mutant strains resistant to 1 γ or more of isoniazid per ml. of medium are highly attenuated for guinea pigs is apparently explained by the observation that this particular mode of attenuation of tubercle bacilli is specifically related to decrease in bacterial catalase activity and its metabolic consequences and, thus, only indirectly related to isoniazid resistance. Several examples of strains resistant to 1 γ of isoniazid per ml. (but susceptible to 10 γ of isoniazid per ml.) and of some strains resistant to 10 γ of isoniazid per ml. have been shown in these studies and elsewhere (4, 5) to be only slightly or moderately attenuated for guinea pigs. In the present investigations, such strains differ, as a group, from the highly attenuated, isoniazid-resistant strains only in their ability to manifest more or less catalase activity *in vitro*.

Adequate bacterial catalase activity, as an essential requirement for bacterial virulence, is not unique to tubercle bacilli. That high catalase production may be one of the prerequisites for full virulence of *Bacillus anthracis* is suggested by the studies of Nungester (22); for *Brucella*, by Merz (23) and Huddleson (24); and for *Pasteurella pestis*, by Rockenmacher (25). The biochemical interpretation of the need of an aerobic parasite for adequate endogenous catalase in order to multiply progressively in certain hosts is not clear. For example, with particular reference to tuberculous disease, it seems fair to assume that the parasites, except in well-aerated, autolyzing tissue such as open pulmonary cavities, multiply predominantly in mononuclear phagocytes, which themselves manifest high catalase activity (26). Possibly, like many enzymes, including the closely related heme-protein, peroxidase (27), catalase is not simply in solution in the cytoplasm of these host cells, but is distributed in granules. In reality, it is probably so anatomically removed, as it were, from the bacterial cells that it cannot play the growth-supportive role which these studies show it can manifest in solution *in vitro*.

Emphasis in the above discussion has been on catalase as a possible growth-supportive for catalase-deficient strains *in vivo*. The concentrations of free inorganic iron or of hemin are surely too low, both intracellularly and extracellularly, in nonautolyzing, normal or inflammatory, tissues to serve as growth-supportives for catalase-deficient microbes *in vivo*. However, the possible role of pyruvic acid, which, as described in these studies, has a striking growth-supportive effect on catalase-negative strains of tubercle bacilli, deserves special attention. The concentration of pyruvate required *in vitro* for its growth-supportive effect is within its physiologic concentration limits in normal tissue. Indeed, to this substance may be attributed the ability of catalase-negative

strains of tubercle bacilli often to initiate multiplication in the tissues of guinea pigs and rabbits, as described here and by Barnett, Bushby, and Mitchison (4), and by Karlson and Ikemi (7), only to stop multiplying when a certain level or type of inflammatory reaction has supervened. It seems reasonable to assume that, in the sufficiently "inflamed" focus, low oxygen tension favors the immediate reduction of any pyruvic acid to lactic acid, which has no growth-supportive activity whatsoever for catalase-negative tubercle bacilli.

The striking difference between the virulence of the catalase-negative strains for guinea pigs (and, in the case of the bovine strain, for rabbits also), on the one hand, and for CF_1 mice, on the other hand, deserves special comment. Identical findings have been reported by Karlson and Ikemi (7) with an isoniazid-resistant strain of H37Rv. It seems justifiable to assume that the failure of mice, in contrast with guinea pigs and rabbits, to develop tuberculin hypersensitivity has a significant effect on the type of inflammatory reaction elicited by multiplying tubercle bacilli and, therefore, on the biochemical environment provided for their subsequent multiplication.

Suffice it to suggest that tuberculin-type hypersensitivity may play a dominant role in determining the peculiarities of the host-parasite relationship in infections with catalase-negative strains of isoniazid-resistant tubercle bacilli.

In conclusion, it should be emphasized that genotypic loss of virulence associated with genotypic loss of susceptibility to the antimicrobial activity of a drug has not been demonstrated except in the tubercle bacillus-isoniazid system. Moreover, the biochemical mechanism of this phenomenon has been elucidated only in so far as the loss of virulence is shown by these studies to reflect a genotypic loss of catalase activity.

SUMMARY

It has been shown that the special growth requirements of certain isoniazid-resistant mutants of tubercle bacilli, most clearly demonstrated in certain well-defined media containing added manganese (0.2 γ per ml.), are intimately related to their genetic deficiency in catalase activity. The most active growth-supportive agents have catalase or catalase-like activity: beef liver catalase, hemin, serum albumin, and alpha-keto acids. Inorganic iron has also been shown to have definite but weak growth-supportive activity for such strains.

Similarly, pathogenicity studies in guinea pigs and rabbits have revealed that the attenuation of certain isoniazid-resistant mutants of tubercle bacilli is also directly related to their deficiency in catalase activity. On the one hand, all strains encountered which were completely negative in tests for endogenous catalase activity were unable to multiply progressively in these hosts, and sometimes were unable even to initiate multiplication. On the other hand, all of the catalase-positive, isoniazid-resistant mutants of virulent tubercle bacilli studied (most of which were susceptible to 10 γ of isoniazid per ml.) were only slightly or moderately attenuated, regardless of their levels of resistance to isoniazid (1 γ or more of isoniazid per ml.)

Mice of the CF_1 strain were shown to be susceptible to air-borne infection

with the three different catalase-negative laboratory strains to which these animals were exposed, and it was pointed out that these animals, in contrast to guinea pigs and rabbits, fail to acquire tuberculin hypersensitivity.

A possible biochemical explanation has been offered for the failure of catalase-deficient strains of tubercle bacilli to continue multiplication in certain inflammatory foci.

SUMARIO

Estudios Relativos a la Isoniacida y los Bacilos Tuberculosos: II. Los Requisitos del Desarrollo, las Actividades Catalásicas y las Propiedades Patógenas de las Mutantes Isoniacido-Resistentes

Está demostrado que los requisitos especiales del desarrollo de ciertas mutantes isoniacido-resistentes de los bacilos tuberculosos, manifestados más claramente en ciertos medios bien definidos a los que se agregado manganeso (0.2 γ por ml.) están íntimamente enlazados con su deficiencia genética en actividad catalásica. Los agentes más activos en apoyo del desarrollo están dotados de actividad catalásica o catalasoidea: catalasa de hígado de bovino, hemina, seroalbúmina y ácidos alfa-quetónicos. Además, se ha demostrado que el hierro inorgánico posee una actividad bien definida, pero débil, para el sostén del desarrollo de esas cepas.

En forma semejante, estudios de la patogenicidad en cobayos y conejos han revelado que la atenuación de ciertas mutantes isoniacido-resistentes de los bacilos tuberculosos está también relacionada directamente con la deficiencia de las mismas en actividad catalásica. Por un lado, todas las cepas observadas que resultaron absolutamente negativas en las pruebas de actividad catalásica endógena se mostraron incapaces de multiplicarse progresivamente en dichos huéspedes y a veces hasta incapaces de iniciar la multiplicación. Por otro lado, todas las mutantes catalasa-positivas isoniacido-resistentes de los bacilos tuberculosos virulentos estudiados (la mayor parte de las cuales eran sensibles a 10 γ de isoniacida por ml.) no mostraron más que atenuación leve o moderada, independientemente de sus índices de resistencia a la isoniacida (1 γ o más de isoniacida por ml.).

Ratones de la cepa CF_1 se mostraron susceptibles a la infección aérea con las tres distintas cepas catalasa-negativas de laboratorio a las que estuvieron expuestos esos animales, apuntándose que éstos, en contraposición a los cobayos y los conejos, no adquieren hipersensibilidad tuberculínica.

Ofrécese una posible explicación bioquímica de la razón porqué las cepas catalasa-deficientes de los bacilos tuberculosos no continúan multiplicándose en ciertos focos inflamatorios.

RESUME

Etude sur l'isoniazide et les bacilles tuberculeux: II. Les conditions du développement, l'action de la catalase et les propriétés pathogènes des mutants isoniazide-résistants

On a découvert que les conditions particulières du développement de certains mutants résistants de bacilles tuberculeux, clairement démontrées dans certains milieux bien définis renfermant du manganèse (0, 2 γ par ml.), sont intimement en rapport avec leur déficience génétique en ce qui concerne l'activité de la catalase. Les agents les plus actifs favorisant la croissance disposent de l'action de la catalase ou d'une action analogue: catalase du foie de boeuf, hémine, sérumalbumine et acides alpha-céto. Le fer inorganique a également fait preuve d'une action stimulante faible, mais définie, sur la croissance de ces souches.

De même, les études sur la pathogénicité effectuée chez le cobaye et le lapin ont révélé que, chez certains mutants isoniazide-résistants du bacille tuberculeux, l'atténuation est également en rapport direct avec une déficience de l'activité de la catalase. D'une part, toutes les souches rencontrées, chez lesquelles les tests d'activité de la catalase endogène

étaient complètement négatifs, étaient incapables de multiplication progressive chez ces hôtes, voire même d'un début de multiplication. D'autre part, tous les mutants isoniazide-résistants de bacilles tuberculeux virulents (la plupart étant sensible à 10γ d'isoniazide par ml.) n'étaient que légèrement ou modérément atténués quel qu'ait été le degré de résistance à l'isoniazide (1γ ou plus d'isoniazide par ml.)

Les souris de la souche CF_1 montraient une sensibilité à l'infection d'inhalation quand elles étaient exposées à trois différentes souches catalase-négatives produites au laboratoire; les auteurs signalent que ces animaux, au contraire des cobayes et des lapins, n'acquéraient pas l'hypersensibilité à la tuberculine.

Une explication biochimique possible est proposée en ce qui concerne l'arrêt de la multiplication des souches de bacilles tuberculeux déficientes en catalase dans certains foyers inflammatoires.

Acknowledgment

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STUDIES ON THE GROWTH OF MYCOBACTERIA[1, 2]

II. The Effect of Oxygenation and Aeration on the Growth Pattern of Mycobacteria

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INTRODUCTION

Fisher and Kirchheimer (1) have recently shown that mycobacterial growth in dispersing media is distinguished by a prolonged arithmetically linear growth which follows the logarithmic growth phase.

Neither exhaustion of nutrients nor accumulation of toxic products in the medium are responsible for the occurrence of arithmetic linear growth (2).

It was therefore suspected that oxygen exhaustion in the medium might be the cause of this phenomenon.

The work reported here was carried out in order to test the validity of this assumption.

MATERIALS AND METHODS

The culture medium employed in this study had the following composition: asparagine, 0.5 per cent; monopotassium phosphate, 0.5 per cent, potassium sulfate, 0.05 per cent; magnesium citrate, 0.15 per cent; and Tween® 80, 0.02 per cent. The pH was adjusted to 7.0 and, following autoclaving, filtration-sterilized bovine albumin (fraction V, Armour) and glucose were added to give a final concentration of 0.2 and 0.5 per cent, respectively.

Aeration and oxygenation experiments: Forty-milliliter aliquots of the above medium contained in 28 by 128 mm-Pyrex® test tubes were inoculated with one of the following mycobacterial strains: *Mycobacterium tuberculosis* var. *hominis* strain H37Ra, *Mycobacterium tuberculosis* var. *bovis* strain BCG, and the saprophytic *Mycobacterium sp.* strain 607. The inocula were made from cultures of these organisms in a medium identical with the one used in the experiments.

Following inoculation, the cultures were incubated in a 37°C. water bath. Aeration or oxygenation was accomplished by passing either air from an air pressure line or oxygen from a tank through sterile water into a glass reservoir. From this, the gas was distributed via polyethylene tubings through a small-bore capillary pipette which penetrated through a rubber stopper to the bottom of the culture medium. Each rubber stopper was provided with a gas outlet hole which was protected with a nonabsorbent cotton plug. The rate of gas flow through the culture medium was adjusted in such a manner that 0, 50,100, or 200 bubbles per minute were delivered into the respective cultures.

In some of these experiments, aeration or oxygenation was commenced immediately following inoculation of the culture medium and was maintained either throughout the experiment or was discontinued before the experiment was ended. In other instances, aeration or oxygenation was started after the culture had passed through the logarithmic growth phase.

Growth was measured turbidimetrically, using the Coleman model 7 photonephelometer, at either twelve- or twenty-four-hour intervals, depending on the rate of growth of the

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particular strain. For each growth measurement, 10 ml. aliquots of the well-shaken culture were removed from the culture tubes, placed into sterile optical cuvettes (Colemen "7-304"), and the optical density was determined in the photonephelometer. The samples were then replaced into their respective tubes.

Growth under reduced oxygen tension: These experiments were carried out by inoculating 7 ml. aliquots of the above medium contained in the aluminum-capped optical cuvettes (Colemen "7-304") with either the human strains H37Rv, H37Ra, or with the bovine strain BCG of the tubercle bacillus.

Following inoculation of the medium, the cuvettes were placed into Brewer jars with a capacity of 2,300 ml. The air then was exhausted from the jars by means of a hy-vacuum pump. Carbon dioxide from a tank was then allowed to enter, to give a constant amount of 5 volume per cent in all Brewer jars. Oxygen was added from a tank to provide 7, 9, 12, 15, and 18 volume per cent, respectively. Sufficient nitrogen was then added to re-establish atmospheric pressure in the Brewer jars. Additional cultures of each organism were placed into Brewer jars containing air at atmospheric pressure. All cultures were incubated at 37°C. in an air incubator.

Growth measurements were carried out daily by removing the cuvettes from the jars for determination of the optical density in the photonephelometer. The cultures then were replaced into the Brewer jars, and the respective gas mixtures were re-established.

Arithmetic and semilogarithmic plots of the nephelometer measurements were made for each culture. The generation times (*GT*) of the organisms were calculated from their respective logarithmic growth rate constants according to the equation $GT = \frac{\log 2}{K}$, where K represents the rate constant of the logarithmic growth. The arithmetic growth rates (*Ra*) were calculated from numerical density values during the arithmetic linear part of the curve by use of the equation $Ra = \frac{N_2 - N_1}{t}$. N_2 is the optical density in "nephelos"[3] units at the end of time interval t. N_1 is the optical density in "nephelos" units at the beginning of time interval t.

RESULTS

The results of the aeration and oxygenation experiments showed that, when aeration or oxygenation of the medium was commenced immediately following inoculation of the organisms and maintained thereafter, the growth proceeded logarithmically during a prolonged period of time without the occurrence of arithmetic linear growth. Strain 607 of *Mycobacterium sp.* required a bubbling rate of 100 per minute to achieve this. The two mammalian strains required a bubbling rate of only 50 per minute.

The effect of continued oxygenation is illustrated in figure 1. From this graph it can readily be seen that the oxygenated culture of *M. tuberculosis* var. *hominis* strain H37Ra grew logarithmically for a prolonged period of time. The culture which was not oxygenated, on the other hand, showed prolonged arithmetic linear growth following a relatively short period of logarithmic growth. In addition, the slope of the straight-line portion of the semilogarithmic plot shows that oxygenation did not accelerate the logarithmic rate of growth above that typical for the organism growing under ordinary atmospheric conditions. Identical results were obtained with all organisms used in this study.

[3] The "nephelos" is the unit of measurement of optical density on the scale of the Coleman model 7 photonephelometer and, under the conditions of the present experiment, corresponds to approximately 500,000 mycobacterial cells per ml.

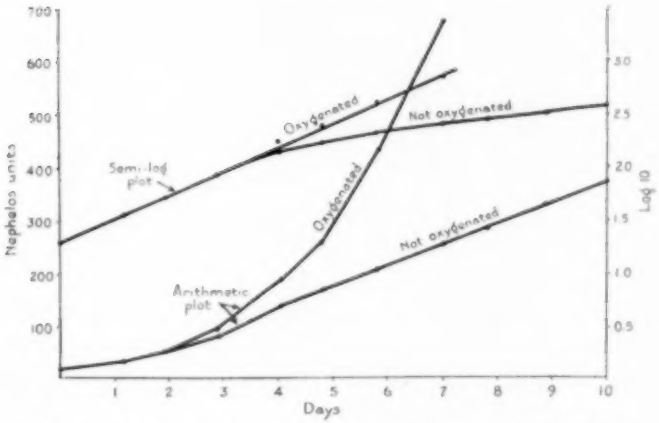

FIG. 1. Arithmetic and semi-logarithmic plots of nephelometric measurements of the growth of *Mycobacterium tuberculosis* var. *hominis* H37Ra in oxygenated and in non-oxygenated medium.

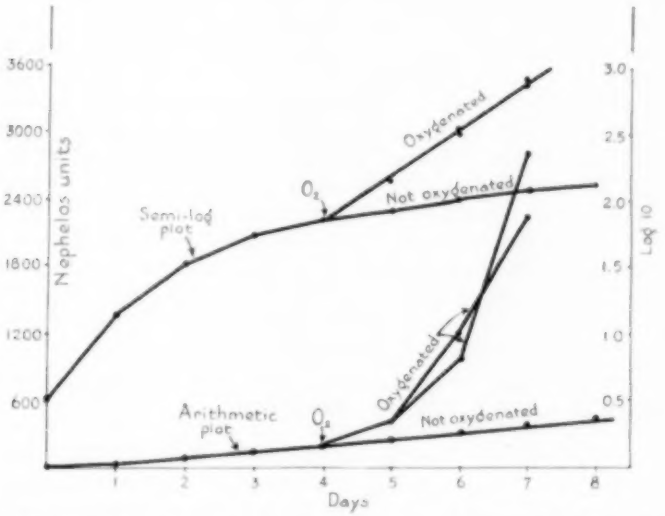

FIG. 2. The effect of oxygenation during arithmetic linear growth on the growth pattern of *Mycobacterium sp.* strain 607.

The effect of oxygenation initiated during arithmetic linear growth is shown in figure 2. In this experiment, oxygenation was initiated at a rate of 100 and 200 bubbles per minute on the fourth day of growth of *Mycobacterium sp.* 607. At this time the growth was arithmetically linear and continued that way in the culture which was not oxygenated. The other culture on oxygenation imme-

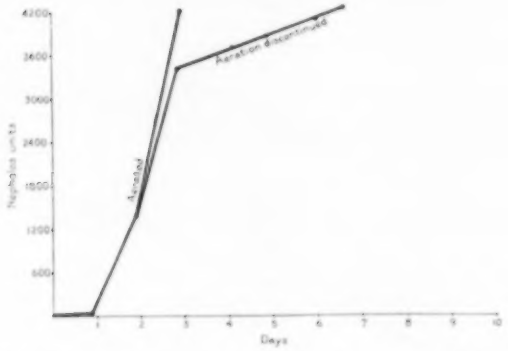

FIG. 3. Growth pattern resulting from discontinuation of oxygenation during logarithmic growth of *Mycobacterium sp.* strain 607.

diately resumed logarithmic growth. Identical results were obtained with cultures of the mammalian tubercle bacilli.

Aerated cultures of mycobacteria which had attained a relatively high cell concentration and were growing logarithmically immediately grew arithmetically when aeration was discontinued. This is shown in figure 3, which is a plot with arithmetic density values on the ordinate.

The results of the studies on mycobacterial growth under reduced oxygen tensions showed that, with decreasing oxygen tensions, the generation times of the different organisms became increasingly longer, until eventually logarithmic growth did not take place at all. Growth in these circumstances was arithmetically linear from the beginning. In the case of the BCG strain of *M. tuberculosis* var. *bovis*, this condition occurred in the presence of 12 volume per cent of oxygen. Strains H37Rv and H37Ra of the human variety of *M. tuberculosis* were capable of logarithmic growth under this condition. At 9 volume per cent of oxygen, only the latter strain grew logarithmically, but logarithmic growth ceased when the oxygen tension was reduced to 7 per cent.

In table 1 the effect of reduced oxygen tensions on the generation times of the 3 organisms used in this study is summarized.

TABLE 1

THE GENERATION TIMES IN HOURS OF STRAINS BCG, H37Rv, AND H37Ra OF MYCOBACTERIUM TUBERCULOSIS GROWING UNDER VARIOUS OXYGEN TENSIONS

Strain	Oxygen Tension				
	Atmospheric	18 Per Cent	15 Per Cent	12 Per Cent	9 Per Cent
BCG	27	36	40	N.L.*	N.L.
H37Rv	21	21	30	30	N.L.
H37Ra	35	34	35	34	47

* N.L. = No logarithmic growth.

TABLE 2

THE ARITHMETIC GROWTH RATES OF STRAINS BCG, H37Rv, AND H37Ra OF MYCOBACTERIUM TUBERCULOSIS GROWING UNDER VARIOUS OXYGEN TENSIONS

Strain	Oxygen Tension				
	Atmospheric	18 Per Cent	15 Per Cent	12 Per Cent	9 Per Cent
BCG	6.60	1.95	1.87	1.16	0.83
H37Rv	6.66	2.58	1.58	1.04	0.66
H37Ra	1.33	1.33	1.16	0.70	0.20

The effect of reduced oxygen tensions on the arithmetic linear growth of the 3 organisms is summarized in table 2 by presenting the arithmetic rates at the various oxygen tensions. The results clearly show that the arithmetic rates for each strain decreased with decreasing oxygen tension.

DISCUSSION

As reported previously (1), arithmetic linear growth is a definite phase of growth of mycobacteria when cultivated under ordinary conditions in a liquid medium with a dispersing agent. During this growth phase the increase in bacterial density exists as a straight-line function of time and it follows a typical logarithmic growth phase. Recently, Fenner and Leach (3) have shown that this pattern of growth also is apparent when growth measurements are based on enumeration of "viable units" rather than on turbidimetric studies.

It does not appear that either exhaustion of nutrients or accumulation of toxic products are responsible for this type of growth. Studies by Fisher and Kirchheimer (2) have shown that culture medium which had been filtered after arithmetic linear growth of mycobacteria had taken place again will support logarithmic growth of an inoculum made from the organisms which had grown in this medium before filtration. In addition, if the bacterial density in the supernatant of a liquid culture in the arithmetic growth phase is decreased by centrifugation, growth in the supernatant again will pass through a logarithmic growth phase.

That the amount of growth produced by tubercle bacilli is within limits proportional to the oxygen tension in the atmosphere of incubation has been shown previously by Novy and Soule (4) and by Corper, Lurie, and Uyei (5) and others.

The results of the present experiments indicate that arithmetic linear growth occurs if the amount of oxygen present in the medium is no longer sufficient to support logarithmic growth of the culture. Recently Volk and Myrvik (6) have stated that the concept of arithmetic linear growth would involve some pattern of reproduction and growth not readily explained in terms of binary fission. There is, however, no morphologic evidence to substantiate such a statement. In addition, an arithmetic linear growth pattern would result if the number of organisms in the culture multiplying during each time interval remained con-

stant. It is not unreasonable to assume that only the upper strata of the medium would contain enough oxygen to support multiplication of a given number of organisms which happen to be present in this zone. It is therefore in order to explain the mechanism of arithmetic linear growth, not necessary to assume a process of multiplication other than binary fission.

It has been shown in this laboratory that constant shaking of mycobacterial cultures in a dispersing medium also prolongs the logarithmic growth phase and tends to eliminate the occurrence of arithmetic linear growth. In the publication quoted above, Volk and Myrvik (6) reported that they had succeeded in eliminating arithmetic linear growth of *M. tuberculosis* var. *bovis* strain BCG by providing adequate aeration by continuously shaking the liquid cultures. However, the graph published by these writers does not support their claim. On the contrary, from their graph it can be seen that logarithmic growth of the shaken culture occurred only for the usual limited period of time and thereafter declined in a manner characteristic of arithmetic linear growth.

It is worth noting here that growth at reduced oxygen tensions did not reveal a relatively greater growth inhibition of the avirulent H37Ra strain in comparison with the virulent H37Rv strain of the human tubercle bacillus. Guy, Raffel, and Clifton (7), using a different method of oxygenation and growing their organisms at an oxygen tension of only 1 volume per cent, recently reported a much greater growth inhibition of the H37Ra strain than of the H37Rv strain.

SUMMARY

Quantitative growth experiments were carried out in order to elucidate the conditions responsible for the occurrence of arithmetic linear growth of mycobacteria in liquid dispersing media.

The results show that continuous aeration and oxygenation of the medium results in logarithmic growth for a prolonged period of time without the occurrence of arithmetic linear growth. The characteristic rate of growth, however, is not changed under these conditions. If aeration or oxygenation is discontinued after the culture has attained a critical cell concentration, arithmetic linear growth will recur. It also is possible to re-establish logarithmic growth by aeration or oxygenation of a culture in the arithmetic growth phase.

Measurements of mycobacterial growth under reduced oxygen tensions show that, as oxygen tension is decreased, the generation time of the organism increases. There is also a progressive decrease of the arithmetic rate of growth under these conditions. None of the organisms used in this study were capable of logarithmic growth at an oxygen tension of 7 volume per cent. Under this condition, growth was entirely arithmetically linear.

SUMARIO

Estudios del Desarrollo de las Micobacterias: II. El Efecto de la Oxigenación y la Aereación sobre el Patrón de Desarrollo de las Micobacterias

Estos experimentos sobre el desarrollo cuantitativo tenían por fin dilucidar los factores causantes de la ocurrencia del crecimiento en progresión aritmética de las micobacterias en medios dispersantes líquidos.

Los resultados demuestran que la continua aereación y oxigenación del medio obtiene crecimiento logarítmico durante un período prolongado de tiempo sin la ocurrencia de crecimiento en progresión aritmética. En esas condiciones, no varía, sin embargo, la velocidad del desarrollo. Si se discontinúan la aereación o la oxigenación después que el cultivo ha alcanzado una concentración celular crítica, recurrirá el crecimiento en progresión aritmética. También es posible restablecer el crecimiento logarítmico por la aereación o la oxigenación de un cultivo en la fase de crecimiento aritmético.

Las mediciones del desarrollo micobacteriano bajo tensiones reducidas de oxígeno revelan que, al disminuir estas tensiones, aumenta el tiempo de generación del microbio. Obsérvase además una disminución progresiva del índice aritmético de desarrollo en esas condiciones. Ninguno de los microbios usados en este estudio se mostró capaz de desarrollo logarítmico a una tensión de oxígeno de 7 por ciento. En estas condiciones, el crecimiento fué absolutamente en progresión aritmética.

RESUME

Etude sur la croissance des mycobactéries: II. Effet de l'oxygénation et de l'aération sur les modalités de la croissance des mycobactéries

Des expériences de croissance quantitative ont été poursuivies pour élucider les conditions responsables de l'occurence de la croissance linéaire arithmétique des mycobactéries dans les milieux liquides de dispersion.

Les résultats montrent que l'aération et l'oxygénation continues du milieu déterminent la croissance logarithmique pendant une période prolongée, sans occurence de la croissance linéaire arithmétique. La vitesse caractéristique de la croissance n'est cependant pas modifiée dans ces conditions. Si l'aération ou l'oxygénation est imterrompue alors que la culture a atteint le point de concentration optimum, la croissance linéaire arithmétique reprendra. Il est également possible de réétablir la croissance logarithmique par l'aération ou l'oxygénation d'une culture au cours de la phase de croissance arithmétique.

Les déterminations de la croissance mycobactérienne, effectuées dans les conditions de tension d'oxygène réduite montrent que, au fur et à mesure que la tension de l'oxygène s'abaisse, le temps de génération de l'organisme l'élève. Dans ces conditions, il y a également un abaissement progressif de la vitesse arithmétique de croissance. Aucun des organismes utilisés dans cette étude n'a été capable de présenter une croissance logarithmique lorsque la tension d'oxygène atteignait 7 volumes pour cent. Dans cette condition, on observait exclusivement la croissance linéaire arithmétique.

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SOME EFFECTS OF PNEUMOPERITONEUM ON RESPIRATION IN PATIENTS WITH PULMONARY TUBERCULOSIS[1, 2]

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With the technical assistance of Betty L. Vestal

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INTRODUCTION

Although artificial pneumoperitoneum enjoys wide popularity in the treatment of pulmonary tuberculosis, there have been few attempts to establish the degree to which this procedure collapses the lung. Indeed, at the time the present study was begun, the work of Wright, Place, and Princi (1) was the only analysis of lung volumes in nonemphysematous patients which indicated whether or not this therapeutic measure is followed by the changes it is intended to produce. There has since appeared an additional comprehensive comparison of the volumetric changes associated with pneumoperitoneum and with pregnancy in tuberculous women (2). The data from the present study of tuberculous men in the military age group are in most instances a confirmation and in some respects an extension of these earlier independent reports.

In order to be effective, a collapse procedure must reduce the volume of air in the lungs when the patient is in the position and at the level of activity selected for his treatment. As bed rest remains a cornerstone of tuberculosis therapy in most clinics, it is essential that pneumoperitoneum diminish the inflation of the lungs during quiet breathing in the supine position. Therapy is therefore directed toward the reduction of that volumetric compartment comprising the functional residual volume (F.R.V.). Assuming that the quantity of blood and lymph in the thorax remains relatively unaffected by pneumoperitoneum, a fall in functional residual volume signifies the displacement of air from the lungs and, consequently, less pulmonary distention. This effect should result from cephalad displacement of the diaphragm. In order to determine these relationships, this study includes pre- and postpneumoperitoneum recordings of diaphragmatic position and measurements of lung volumes with the patient supine. In addition, data concerning the elimination of nitrogen from the lungs during oxygen breathing and on the relationship between alveolar ventilation and perfusion are included.

MATERIALS AND METHODS

The patients were 33 men in the Armed Forces who were hospitalized for pulmonary tuberculosis at the U. S. Naval Hospital, St. Albans. The mean age was 26.5 years; only

[1] From the Cardiopulmonary Function Laboratory, U. S. Naval Hospital, St. Albans, New York.

[2] This study was assisted by a contract between the Office of Naval Research, Department of the Navy, and the State University of New York, NR 105 051.

TABLE 1

CLINICAL AND LUNG VOLUME DATA ON 33 MALE TUBERCULOUS PATIENTS STUDIED BEFORE AND AFTER TREATMENT WITH PNEUMOPERITONEUM

Patient Number	Age years	Body Surface Area, m^2	Severity of Tuberculosis	Weeks of Pneumoperitoneum	Functional Residual Volume (Liters)			Vital Capacity (Liters)			Residual Volume (Liters)			Total Lung Volume (Liters)		
					Pre	Post	Per Cent Change	Pre	Post	Per Cent Change	Pre	Post	Per Cent Change	Pre	Post	Per Cent Change
0470	22	1.77	Minimal	5	2.32	2.03	−13	4.36	4.34	−1	1.32	1.01	−24	5.68	5.35	−6
0474	20	1.66	Moderately advanced	5	2.97	1.58	−47	3.96	4.14	+5	2.16	0.61	−72	6.20	4.75	−6
0475	40	1.86	Moderately advanced	5	4.62	2.65	−43	4.45	4.37	−2	3.57	1.69	−53	8.03	6.06	−25
0476	23	1.72	Minimal	6	4.06	2.34	−42	4.82	4.41	−9	2.53	1.45	−43	7.35	5.85	−20
0477	22	1.94	Moderately advanced	6	2.43	2.22	−9	4.94	4.77	−3	1.43	1.15	−20	6.38	5.92	−7
0486	37	1.74	Moderately advanced	3	3.17	2.29	−28	4.50	4.72	−8	2.10	1.32	−43	6.60	6.04	−20
0487	25	1.81	Minimal	8	2.45	1.98	−19	5.17	5.48	+6	1.22	1.12	−8	6.39	6.60	+3
0489	24	1.81	Moderately advanced	8	2.53	1.87	−26	4.05	4.33	+7	1.25	1.37	+10	5.30	5.69	+7
0496	21	1.93	Minimal	10	2.33	1.93	−17	4.10	4.56	+11	1.42	1.15	−19	5.52	5.71	+3
0504	24	1.96	Minimal	7	3.10	2.76	−11	6.11	6.46	+6	1.59	1.44	−9	7.70	7.90	+3
0511	18	1.83	Moderately advanced	6	2.27	1.66	−27	4.78	4.62	−3	1.27	1.01	−20	6.04	5.63	−7
0514	23	1.94	Far advanced	8	2.53	2.37	−6	4.84	4.01	−17	1.26	1.50	+19	6.09	5.51	−10
0527	26	1.75	Minimal	9	2.61	2.12	−19	4.27	3.74	−12	1.69	1.52	−10	5.96	5.29	−12
0529	21	1.85	Minimal	9	2.86	2.01	−30	4.28	3.62	−15	1.20	0.96	−20	5.48	4.58	−16
0533	32	1.74	Moderately advanced	8	2.39	2.36	−1	4.31	4.33	+1	1.50	1.50	0	5.80	5.83	+1
0538	37	1.92	Moderately advanced	9	4.28	3.79	−11	6.94	6.89	−1	2.31	2.15	−7	9.25	9.04	−2
0544	36	1.86	Minimal	9	3.61	3.04	−16	5.24	5.42	+3	2.30	1.72	−25	7.54	7.14	−5
0558	31	1.75	Moderately advanced	9	2.55	1.69	−34	3.90	2.78	−29	1.84	1.17	−36	5.74	3.95	−31
0559	24	1.74	Moderately advanced	9	2.40	1.90	−21	4.25	4.22	−1	1.46	0.50	−66	5.71	4.72	−17
0560	19	2.03	Moderately advanced	8	3.08	2.64	−14	5.26	5.30	+1	1.96	1.50	−23	7.22	6.81	−6
0562	21	1.81	Moderately advanced	9	1.94	1.40	−28	3.29	3.25	−1	1.07	0.66	−35	4.30	3.91	−9
0566	44	1.77	Moderately advanced	9	2.84	2.18	−23	4.29	4.14	−4	1.86	1.21	−35	6.15	5.35	−13
0569	21	1.71	Minimal	10	2.34	2.01	−14	4.65	4.63	0	1.39	1.03	−26	6.04	5.67	−6
0574	27	1.74	Moderately advanced	11	2.72	1.80	−34	3.28	3.22	−2	1.90	0.95	−50	5.18	4.16	−20
0583	31	1.75	Far advanced	9	2.53	2.17	−14	4.36	3.95	−9	1.28	1.22	−5	5.65	5.17	−9
0637	31	1.58	Moderately advanced	6	3.02	2.48	−18	4.24	4.01	−5	1.94	1.44	−26	6.18	5.45	−12
0638	20	1.91	Far advanced	6	1.65	1.62	−2	3.57	2.81	−21	0.94	1.27	+35	4.51	4.07	−10
0642	27	1.75	Far advanced	6	2.14	1.54	−25	3.14	3.13	0	1.41	0.81	−43	4.55	3.94	−13
0645	28	1.76	Moderately advanced	6	3.14	2.53	−19	5.56	4.92	−12	1.65	1.06	−36	7.21	5.98	−17
0646	23	1.69	Moderately advanced	8	2.63	2.58	−2	5.28	4.97	−6	1.21	1.39	+15	6.50	6.35	−2
0648	27	1.95	Far advanced	6	1.81	1.71	−5	4.07	3.61	−11	0.91	1.12	+23	4.98	4.73	−5
0658	22	1.99	Moderately advanced	7	3.55	2.74	−23	5.65	4.83	−14	2.40	1.87	−22	8.05	6.70	−17
0561	27	1.68	Moderately advanced	8	2.72	1.76	−35	4.18	3.72	−11	1.88	1.04	−45	6.06	4.76	−21
Mean							−20.4			−4.4			−21.6			−10.1
Standard deviation							12.7			8.2			24.0			9.3
CONTROLS																
Mean							−2.8			+0.1			−8.1			−3.6
Standard deviation							3.7			3.5			16.2			8.0

9 patients were older than 30 years (table 1). The studies reported on each patient are the pre- and postpneumoperitoneum measurements of the total lung volume and its subdivisions. None of the tests used are free from some degree of variation due to subjective factors. In order to increase the likelihood that changes observed were reliable, the following precautions were taken:

1. In measuring the functional residual volume (Darling (3)), duplicate determinations were required to check within 200 ml. or 10 per cent in order to be acceptable; the mean of these was used in the tables.

2. The vital capacity was performed three times during combined spirometry and the best effort was selected for use.

3. Differences between pre- and postpneumoperitoneum values were compared with those found in 10 healthy untreated young men (table 1, controls) studied during the same period as the patients and subjected to two studies spaced six to eight weeks apart.

Statistical procedures consisted of calculating the difference between pre- and postpneumoperitoneum (or, in the controls, first and second) studies, dividing this difference by the initial or prepneumoperitoneum value and finding the mean of this per cent change in both patient and control groups.[3] The standard deviation, standard error of the mean, standard error of difference between means, and '*t*' were then computed (4). Significance was attached to differences between the means of the two groups when values of *P* were less than 0.05, i.e., when, according to this analytical procedure, the observed difference between means could be expected to occur in a random sampling less than once in twenty times. All lung volumes were corrected to body temperature, ambient pressure, saturated (BTPS).

The concentration of nitrogen remaining in "alveolar" air was determined as a part of the functional residual volume measurement after the patient had breathed virtually 100 per cent oxygen for seven minutes (index of intrapulmonary mixing of respiratory gases) (3). Comparison was made between the pre- to postpneumoperitoneum change in this concentration among 31 patients exhibiting a fall in functional residual volume of 15 per cent or more (table 2) and the changes among 7 tuberculosis patients and 10 controls whose second study, with one exception, showed a fall in functional residual volume of less than 10 per cent. Thus, the change in the mixing index in a group of 31 who showed a 15 per cent or more fall in functional residual volume was compared with that in a group of 17, all but one of whom demonstrated a fall of less than 10 per cent.

The analysis of ventilation-perfusion relationships in terms of "physiologic dead-space-like" effect and "venous-admixture-like" component (table 3) was patterned after the exposition of Riley and associates (5, 6). Blood gas tensions were measured by the bubble equilibration method (7), and the analysis of expired air was performed with the Scholander apparatus (8).

Blood and gas samples were collected in the post-absorptive state with the patient resting supine. The abbreviations appearing in table 3 are those recommended by a committee of workers in respiratory physiology (9).

$P_{A_{CO_2}}$: partial pressure of carbon dioxide in alveolar air.

BP-47: barometric pressure corrected for water vapor tension of saturated gas at body temperature (37° C.).

$F_{E_{CO_2}}$: concentration of carbon dioxide in expired air.

V_T: tidal volume.

V°_D/BSA: dead space volume corrected for apparatus dead space (75 cc.) divided by the body surface area. Normal values are less than 100 ml.

[3] Applied only to lung volumes. Alveolar nitrogen statistics are based simply on the difference in concentration between the two studies.

TABLE 2

PRE- AND POSTPNEUMOPERITONEUM "MIXING INDEX"* IN 31 TUBERCULOUS PATIENTS SHOWING A FALL IN FUNCTIONAL RESIDUAL VOLUME IN EXCESS OF 15 PER CENT†

Patient Number	Per Cent Fall in Functional Residual Volume	"Alveolar Nitrogen"		Change in "Alveolar Nitrogen" Volumes Per Cent
		Prepneumoperitoneum Volumes Per Cent	Postpneumoperitoneum Volumes Per Cent	
0129	20	1.10	0.80	−0.30
0153	40	2.90	2.00	−0.90
0249	39	1.40	0.80	−0.60
0266	20	0.70	0.70	0
0267	29	0.55	0.45	−0.10
0272	18	2.60	2.00	−0.60
0278	20	1.50	0.92	−0.60
0302	18	0.60	0.65	+0.05
0309	28	1.80	0.81	−0.19
0324	42	1.70	1.40	−0.30
0327	21	2.60	2.10	−0.50
0329	37	1.30	0.60	−0.70
0332	30	0.80	0.40	−0.40
0333	17	1.30	0.50	−0.80
0350	27	1.30	1.10	−0.20
0365	37	0.84	0.80	−0.04
0474	47	0.45	0.62	+0.17
0475	43	2.25	2.16	−0.09
0476	42	0.52	0.47	−0.05
0486	28	1.43	0.82	−0.61
0487	19	0.63	1.26	+0.63
0489	26	5.24	0.86	−4.38
0496	17	0.75	0.60	−0.15
0511	27	0.78	0.41	−0.37
0527	19	0.54	0.26	−0.28
0529	30	0.55	0.58	+0.03
0544	16	0.81	1.41	+0.60
0558	34	2.48	0.52	−1.96
0559	21	2.19	1.67	−0.52
0566	23	1.49	0.78	−0.71
0574	34	0.94	0.81	−0.13
Mean	−28			−0.45
Standard deviation				0.85
CONTROLS				
Mean	−28			+0.11
Standard deviation				0.23

* Concentration of nitrogen in "alveolar" air at the end of a seven-minute period of oxygen breathing.

† Patients 0129 through 0365 are from a previous unpublished study; they are not elsewhere recorded in this report.

TABLE 3

DISTRIBUTION DATA IN 10 PATIENTS RECEIVING PNEUMOPERITONEUM

Patient Number	Age	Body Surface Area	Severity of Tuberculosis	State of Pneumoperitoneum	$P_{A_{CO_2}}$	BP-47	$F_{E_{CO_2}}$	V_T	V^c_D/BSA	$V^c_D/V_T \times 100$	$F_{I_{O_2}}$	$P_{I_{O_2}}$	R	$P_{A_{O_2}}$	$P_{a_{O_2}}$	$\dot{Q}_{VA}/\dot{Q}_t$	Per Cent Fall in Functional Residual Volume
0486	37	1.74	Moderately advanced	Pre	41	703	3.483	497	72	25	20.93	147	1.12	109	90	8*	
		1.73		Post	45	733	2.820	450	97	37	20.93	153	0.87	102	93	4	28
0538	37	1.92	Moderately advanced	Pre	38	710	3.300	559	73	25	20.93	149	0.88	107	95	5	
		1.96		Post	38	719	3.162	553	76	27	20.93	151	0.82	102	92	5	11
0544	36	1.80	Minimal	Pre	42	704	3.261	592	107	33	20.93	147	0.91	102	80	9	
		1.86		Post	44	714	3.686	696	110	30	20.93	149	0.84	99	82	9	16
0558	31	1.74	Moderately advanced	Pre	39	712	3.971	500	46	16	20.93	149	0.89	104	91	6	
		1.76		Post	45	711	3.405	365	53	26	20.93	149	0.81	96	88	4	34
0562	21	1.81	Moderately advanced	Pre	44	712	2.730	300	51	31	20.93	149	0.84	102	93	4	
		1.80		Post	40	711	3.139	364	48	24	20.93	149	0.85	104	94	5	28
0566	44	1.77	Moderately advanced	Pre	47	715	2.962	450	97	38	20.93	150	0.84	96	77	12	
		1.76		Post	43	716	2.805	440	85	34	20.93	150	0.88	104	84	10	23
0574	27	1.74	Moderately advanced	Pre	48	703	3.305	400	75	33	20.93	147	0.92	96	88	4	
		1.72		Post	45	715	2.881	358	69	33	20.93	150	0.86	102	91	5	34
0583	31	1.75	Far advanced	Pre	42	723	2.676	492	109	39	20.93	151	0.83	103	90	6	
		1.78		Post	41	715	3.394	508	101	34	20.93	150	0.84	103	94	4	14
0648	27	1.95	Far advanced	Pre	48	707	3.434	413	66	31	20.93	148	0.82	94	92	2	
		1.87		Post	44	714	3.337	412	61	28	20.93	149	0.84	100	92	4	6
0658	22	1.99	Moderately advanced	Pre	31	707	2.963	809	95	23	20.93	148	0.74	97	84	7	
		1.99		Post	39	711	3.578	585	65	22	20.93	149	0.78	93	80	8	23

* Unreliable because of high R value.

TABLE 4

PRE- AND POSTPNEUMOPERITONEUM CHANGES IN POSITION OF DIAPHRAGM AND IN LUNG VOLUMES IN 18 TUBERCULOUS PATIENTS

Patient Number	Severity of Tuberculosis	Cephalad Displacement of Diaphragm by Pneumoperitoneum*			Per Cent Change in			Movement of Diaphragm on Inspiration† (mm.)		Inspiratory Capacity/Vital Capacity × 100	
		Midposition	Inspiration	Expiration	Functional Residual Volume	Total Lung Volume	Residual Volume	Before Pneumoperitoneum	After Pneumoperitoneum	Before Pneumoperitoneum	After Pneumoperitoneum
0476	Minimal	½	1½	3	−42	−20	−43	83	83	78	83
0487	Minimal	1½	½	2	−19	+3	−8	86	86	72	84
0489	Moderately advanced	2	1	2	−26	+7	+10	75	86	70	82
0504	Minimal	1	½	1½	−11	+3	−9	67	69	68	74
0511	Moderately advanced	1½	1½	2	−27	−7	−20	78	64	69	82
0527	Minimal	1	0	1	−19	−12	−10	83	93	71	80
0529	Minimal	2½	1½	1½	−30	−16	−20	43	63	53	73
0538	Moderately advanced	1½	½	1	−11	−2	−7	38	60	72	76
0558	Moderately advanced	1½	2	1½	−34	−31	−36	73	100	72	84
0559	Moderately advanced	1	1	1½	−21	−17	−65	50	37	78	67
0560	Moderately advanced	1	½	1	−14	−6	−23	79	82	73	76
0562	Moderately advanced	1½	½	1½	−28	−9	−35	71	96	74	79
0566	Moderately advanced	1	0	0	−23	−13	−34	67	90	70	74
0569	Minimal	1½	1	½	−14	−6	−26	47	88	62	64
0637	Moderately advanced	1	1	½	−18	−12	−26	51	90	65	74
0638	Far advanced	1½	1	1	−2	−10	+35	71	90	73	77
0648	Far advanced	1½	½	1	−6	−5	+23	67	84	68	80
0658	Moderately advanced	1	½	1	−23	−17	−22	73	85	71	76
Mean					−20	−9	−18	67	80	70	77

* Figures indicate displacement in terms of the width of a vertebral body.

† Figures are the ratio: millimeters from midposition to inspiratory position/millimeters from expiratory position to inspiratory position × 100.

V^{n}_{D}/V_{T}: ratio of dead-space volume to tidal volume in per cent. Normal values are less than 30.

F_{IO_2}: concentration of oxygen in inspired air.

P_{IO_2}: partial pressure of oxygen in inspired air.

R: the gas exchange ratio (R.Q.).

P_{AO_2}: oxygen partial pressure in alveolar gas.

P_{aO_2}: oxygen tension in brachial artery blood.

$\dot{Q}_{va}/\dot{Q}_{t}$: the ratio of venous admixture to total cardiac output (normally less than 6 per cent).

The pre- and postpneumoperitoneum position of the diaphragm was recorded by right lateral roentgenograms with the patient supine (table 4). Three roentgenograms were taken at each examination. These consisted of a film with the diaphragm in the midposition, a second at the end of maximum inspiration, and a final film exposed with the diaphragm in the maximum expiratory position. In each instance the position of the diaphragm was verified by a simultaneously recorded combined spirometry tracing. The intersection of the more elevated leaf of the diaphragm with the anterior vertebral border was used as the point of reference in measuring positional changes.

An attempt was made to obtain the second study only after a sustained pneumoperitoneum had been established. Air was introduced into the peritoneal cavity to the limit of tolerance twice weekly for the first two or three weeks of its administration and once weekly thereafter. As indicated in table 1, all but 4 patients had received treatment for six or more weeks preceding their second study. Each patient was studied within three days following a refill.

RESULTS

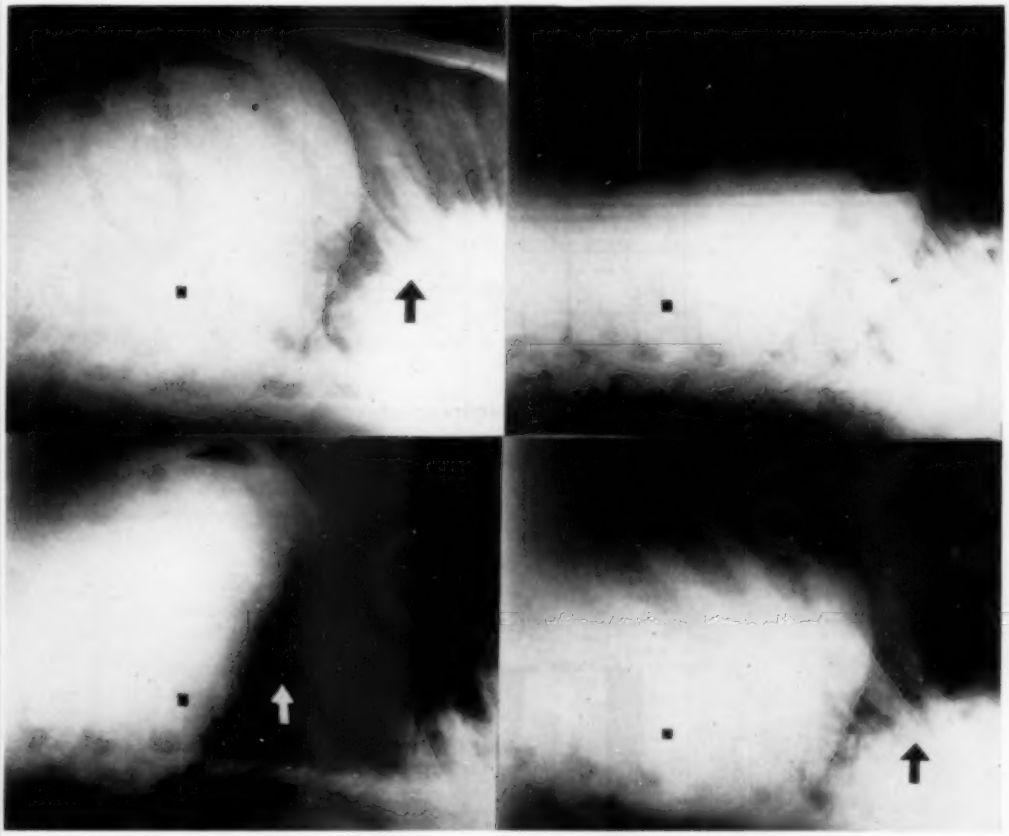
The Effect of Pneumoperitoneum on the Position of the Diaphragm (Table 4, Figure 1*A*)

The midposition level of the diaphragm was displaced cephalad by at least the width of one vertebral body in all except one of 18 patients. With 2 exceptions, the level on maximum inspiration shifted in the same direction and, with one exception, the position on maximum expiration was similarly affected. As can be seen from the percentage changes in the lung volumes corresponding to these three positions (table 4), the measurement of displacement was too crude to be related quantitatively to changes in these volumes.

The caudad movement of the diaphragm from mid- to inspiratory position before treatment was compared with that following pneumoperitoneum. In 14 of the 18 patients, following pneumoperitoneum this movement in millimeters made up a larger proportion of the total stroke from maximum expiration to maximum inspiration. This proportion was expressed as per cent, the mean prepneumoperitoneum value being 67 per cent, compared with 80 per cent following treatment. Correspondingly, in 17 of the 18 patients the per cent of the vital capacity contributed by the inspiratory capacity was increased by pneumoperitoneum, the group mean for the first study being 70 per cent, compared with 77 per cent for the second study (table 4).

The Effect of Pneumoperitoneum on Lung Volumes (Table 1 and Figures 1*B*, 2, and 3)

The *functional residual volume* (F.R.V.) was reduced in each of 33 patients (figure 3*A*); the group mean fall was 20.4 per cent (standard deviation = 12.7)

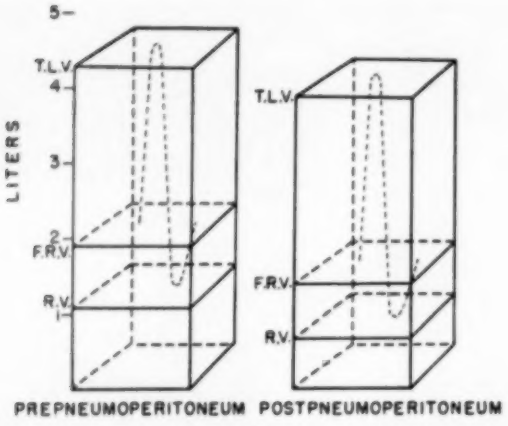


A

FIG. 1A. Patient 0562. Right lateral roentgenograms taken at midposition (prepneumoperitoneum, upper left; postpneumoperitoneum, upper right), at which time lungs contain the functional residual volume; at inspiration (lower left), at which time lungs contain the total lung volume; and at expiration (lower right), when the lungs contain the residual volume. In each film, square marker indicates T-12, arrow shows position of diaphragm after pneumoperitoneum. Note cephalad displacement of diaphragms by treatment; effect on corresponding lung volumes is shown in figure 1*B*.

and the range extended from 1 per cent to 47 per cent. In contrast, the mean fall among the 10 controls was only 2.8 per cent. The likelihood of this difference in means occurring by chance is less than one in 100 (P = less than 0.01).

The *vital capacity* (Vit. Cap.) was adversely affected in 23 of the 33 patients, remained unchanged in 2, and improved in 8 (figure 3*B*). The group mean change was minus 4.4 per cent (standard deviation = 8.2), as compared with plus 0.1 per cent for the controls, a difference in means which is significant (P = less than 0.05).



B

FIG. 1*B*. Lung volumes before and after pneumoperitoneum in patient No. 0562. T. L. V. —total lung volume, F. R. V.—functional residual volume, R. V.—residual volume. The volumetric changes may be compared to displacement of diaphragms at inspiration, mid-position, and expiration indicated in figure 1*A*.

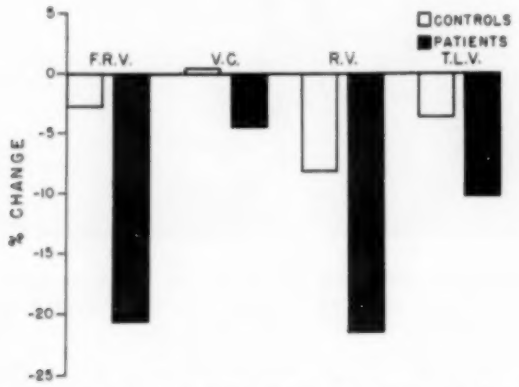


FIG. 2. Mean lung volume changes in control and patient groups. F. R. V.—functional residual volume, V. C.—vital capacity, R. V.—residual volume, T. L. V.—total lung volume.

The residual volume (Res. Vol.) fell in 27 of the 33, was unaffected in one patient, and increased in 5 (figure 3*C*). The group mean change was minus 21.6 per cent (standard deviation = 24), with a range from plus 35 per cent to minus 72 per cent. Surprisingly enough, 8 of the 10 control subjects also showed a fall; the mean change for these 10 subjects was minus 8.1 per cent. Because of this unexpected result and due to the wide scatter of the changes present in both

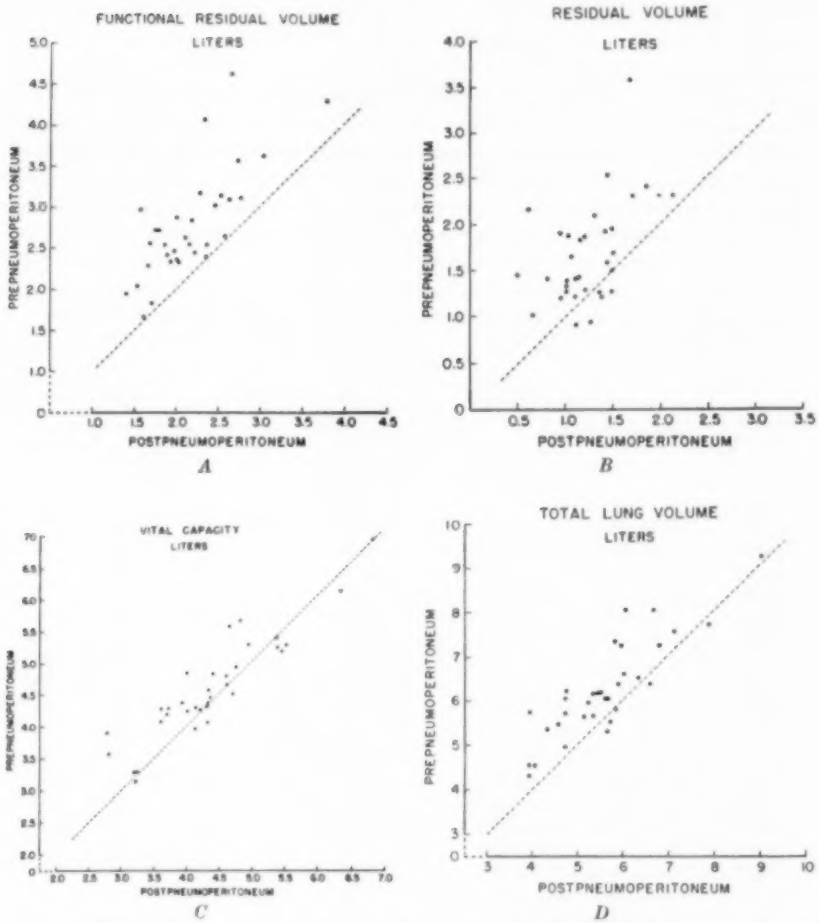


FIG. 3. Pre- and postpneumoperitoneum lung volumes in 33 men with pulmonary tuberculosis. In each figure, broken line indicates position that all dots would occupy if pre- and postpneumoperitoneum values were equal. Note that most dots lie above this line, indicating reduction in volume following pneumoperitoneum.

groups, analysis does not prove these differences between means to be significant statistically (P = more than 0.05). It is believed, however, that, with the use of a larger control group, significance by this statistical approach would be established.

As would be anticipated from effects on vital capacity and residual volume, pneumoperitoneum produced a fall in *total lung volume* in most patients (28 of 33; figure 3*D*). The mean change was minus 10.1 per cent (standard deviation

= 9.3), with a range from plus 7 per cent to minus 31 per cent, as compared with minus 3.6 per cent among the controls. The difference between these two means is significant (P = less than 0.05).

It is apparent, therefore, that each of the principal components of the total lung volume was reduced by pneumoperitoneum in most patients. That the residual and functional residue volumes generally fell more than the vital and inspiratory capacities is indicated by the fact that the ratio residual volume/total lung volume was lowered by pneumoperitoneum in 25 of the 33 patients and the ratio functional residual volume/total lung volume in 28 patients (table 5).

TABLE 5

VOLUMETRIC RATIOS AND THE RELATIONSHIP BETWEEN SEVERITY OF DISEASE AND EFFECTIVENESS OF PNEUMOPERITONEUM IN 33 MEN WITH PULMONARY TUBERCULOSIS

Patient Number	Severity of Tuberculosis	Per Cent of Predicted Vital Capacity	Per Cent Change in Functional Residual Volume	Residual Volume/Total Volume × 100		Functional Residual Volume/Total Volume × 100	
				Pre	Post	Pre	Post
0470	Minimal	97	−13	23	19	41	38
0476	Minimal	111	−42	34	25	55	40
0487	Minimal	118	−19	19	17	38	30
0496	Minimal	91	−17	26	20	42	34
0504	Minimal	133	−11	21	18	40	35
0527	Minimal	101	−19	28	29	44	40
0529	Minimal	95	−30	22	21	52	44
0544	Minimal	121	−16	31	24	48	43
0569	Minimal	105	−14	23	18	39	35
0474	Moderately advanced	96	−47	35	13	48	33
0475	Moderately advanced	108	−43	45	28	58	44
0477	Moderately advanced	107	−9	23	20	38	38
0486	Moderately advanced	111	−28	32	22	48	38
0489	Moderately advanced	88	−26	24	24	48	33
0511	Moderately advanced	109	−27	21	18	38	29
0533	Moderately advanced	102	−1	26	26	41	40
0538	Moderately advanced	159	−11	25	24	46	42
0558	Moderately advanced	93	−34	32	30	44	43
0559	Moderately advanced	96	−21	26	11	42	40
0560	Moderately advanced	113	−14	27	22	43	39
0561	Moderately advanced	101	−35	31	22	45	37
0562	Moderately advanced	75	−28	24	17	45	36
0566	Moderately advanced	109	−23	30	23	46	41
0574	Moderately advanced	75	−34	37	23	53	43
0637	Moderately advanced	104	−18	31	27	49	46
0645	Moderately advanced	130	−19	23	18	44	42
0646	Moderately advanced	118	−2	19	22	40	41
0658	Moderately advanced	123	−23	30	28	44	41
0514	Far advanced	105	−6	21	27	42	43
0583	Far advanced	100	−14	23	24	45	42
0638	Far advanced	77	−2	21	31	37	40
0642	Far advanced	74	−25	31	21	45	39
0648	Far advanced	89	−5	18	24	36	36

The Effect of Pneumoperitoneum on the "Index of Intrapulmonary Mixing"
(Table 2 and Figure 4)

The value used for the index of intrapulmonary mixing is the concentration of nitrogen present in "alveolar" air after the patient has "rinsed" his lungs with virtually 100 per cent oxygen for seven minutes (3). The mean of duplicate determinations of this concentration after pneumoperitoneum was lower than that before treatment in 25 of 31 patients who demonstrated reductions in functional residual volume of 15 per cent or more. In contrast, a fall was found in only 4 of the 10 control subjects and in 2 of 7 pneumoperitoneum patients whose functional residual volume had diminished by less than 10 per cent (a decrease in 6 of 17 subjects). The mean for the former group was minus 0.45 volumes per cent (standard deviation = 0.85) and for the latter plus 0.11 volumes per cent (standard deviation = 0.23). The difference between these means is significant (P = less than 0.05).

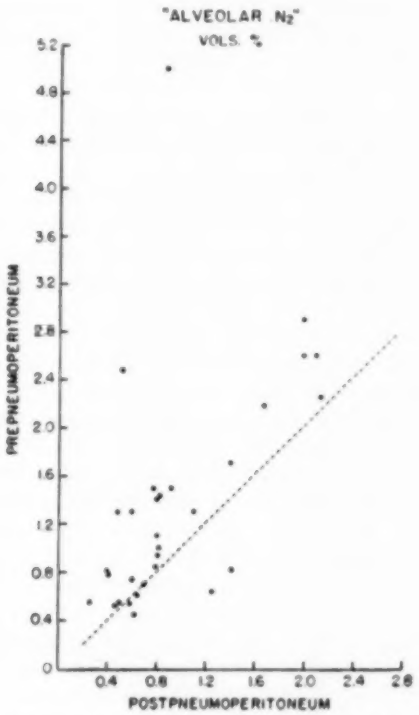


FIG. 4. Pre- and postpneumoperitoneum concentration of nitrogen in "alveolar" air at the end of a seven-minute period of oxygen breathing in 31 men whose treatment produced a fall in functional residual volume of 15 per cent or more. All dots would fall on broken line if pre- and postpneumoperitoneum values were identical. Note that index was lowered by pneumoperitoneum in most instances.

The Effect of Pneumoperitoneum on Ventilation-Perfusion Relationships

As is apparent from table 3, no consistent change in either "physiologic dead-space-like" ($V^{\cdot}_D/V_T$) or "venous admixture-like" ($\dot{Q}_{va}/\dot{Q}_t$) components was evident among 10 patients in whom these measurements were made both before and after pneumoperitoneum; differences between the two studies did not exceed the limits of accuracy of these determinations (10).

DISCUSSION

After the fifth week, pneumoperitoneum given to the limits of tolerance once weekly produced in most patients a cephalad displacement of the diaphragm in the inspiratory, expiratory, and midpositions. Just as these changes have been documented with the patient upright (11), so they were also obtained with the patient resting in bed.

In consequence of this elevation of the diaphragms, the lung volumes corresponding to these three positions were usually reduced. Moreover, the vital capacity was also commonly slightly diminished. Speculations relative to this latter effect would include the thoughts that the diaphragms are handicapped in their maximum stroke when contracting against increased intra-abdominal pressure and, further, that, under the prolonged influence of continuous stretch, these muscles and tendons are unable to acquire their original minimal length. Such reasoning is not at variance with the possibility that the diaphragms and chest wall can perform submaximal rapid movements in an unimpaired or even, as found by Patton and associates (2), in a more effective way. Unpublished data from this laboratory indicate that the maximum breathing capacity is not significantly affected by five to ten weeks of pneumoperitoneum. This is at variance with the deleterious effect noted by others (1, 12).

As reported previously (2, 13), the proportion of the vital capacity contributed by the inspiratory capacity was augmented by pneumoperitoneum. This follows from the fact that, because of their higher midposition levels, the diaphragms after pneumoperitoneum have a caudad stroke which comprises a larger proportion of the total movement from expiration to inspiration (table 4).

The mean reduction in functional residual volume observed among these 33 men (20.5 per cent) is in close agreement with that found by Wright and associates (1) in 18 patients (17.5 per cent) and by Patton and associates (2) in 9 women (17 per cent). As indicated by the former writers, this change is only a fraction of the total collapse engendered by a combination of bed rest and pneumoperitoneum, for the midposition commonly changes by minus 20 to minus 30 per cent (1, 14) with a shift from the upright to the supine position.

The smaller volume of the lungs in the supine midposition implies a new equilibrium between distending and retracting forces within the lung in which the former have been curtailed. Thus, the same bulk of pulmonary tissue is made to fill a smaller space. Correspondingly, a given volume of tidal air is diluted less on inspiration after pneumoperitoneum than it was before, and the total volume of nitrogen in the lungs is diminished. The replacement of this nitrogen by oxygen during the seven-minute test is, therefore, more complete.

The changes in the "index of intrapulmonary mixing", recorded in table 2, may be attributed to these factors rather than to a more uniform or even filling of alveoli, an effect which, too, would result in an improved "mixing index" (15).

The "dead-space-like" volume is contributed by: gas filling spaces lined by tissue across which diffusion of respiratory gases does not occur (airways and unperfused alveoli), and by gas from poorly perfused alveoli (5, 16). It may be concluded from the relative constancy of values recorded in table 3 that pneumoperitoneum and the change in midposition produced by it neither reduce the former compartment nor improve alveolar perfusion to an extent within the range of sensitivity of this determination.

Similarly, "venous-admixture-like" effect is contributed by streams of blood entering the arterial flow from two sources: venous channels (straight right-to-left shunt bypassing the lungs), and poorly ventilated but well-perfused alveoli (5, 16). Pneumoperitoneum would hardly be expected to alter the first of these sources. The absence of a trend in $\dot{Q}_{va}/\dot{Q}_t$ listed in table 3 may therefore be interpreted to signify unchanged ventilation among perfused alveoli. In summary, therefore, the values for "dead-space-like" and "venous-admixture-like" effects reflect no uniform change in the relationship between alveolar perfusion and ventilation in the lungs of the 10 patients included in table 3. It should be observed that only one of these men (No. 0566) demonstrated prominent abnormalities in the distribution of pulmonary blood and gas. These data, therefore, relate to effects of pneumoperitoneum on distribution in patients with ventilation-perfusion relationships which for the most part are within normal limits.

As noted above, the magnitude of change in functional residual volume varied extremely (minus 47 per cent to minus 1 per cent). In accordance with the suggestion of Patton and associates (2) that the effectiveness of pneumoperitoneum may be related to the extent of disease, the present data were examined for a correlation between severity of tuberculosis and per cent fall in functional residual volume (table 5). Five of the patients had *far advanced* disease confined by roentgenographic appearance to the parenchyma and not producing fixation of the diaphragms. Only one of these 5 patients (No. 0642) demonstrated a fall in functional residual volume exceeding the mean value for all patients. In 3 of the 5, the fall was less than 10 per cent; the mean reduction for the group was 10 per cent.

Nine patients had *minimal* disease. In none of these was the fall in functional residual volume less than 10 per cent; their mean change was minus 21 per cent. It should be observed also that 3 of the 5 patients with far advanced disease had a vital capacity less than 95 per cent of predicted (mean = 89 per cent predicted), whereas only one of the 9 patients with minimal tuberculosis had a vital capacity below 95 per cent of predicted (mean = 108 per cent of predicted). These data suggest that the effectiveness of pneumoperitoneum varies inversely with the extent of disease.

In patients such as the 5 referred to above, in whom fixation of the diaphragm did not account for failure of collapse, one wonders if diminished pulmonary

compliance has possibly rendered the lungs unusually resistant to deflation by pressure from below. That extent and nature of disease together with the functional status are not the only factors upon which the outcome of pneumoperitoneum is predicated, however, is shown by patients Nos. 0477, 0533, and 0646, each of whom had moderate disease, freely movable diaphragms, and normal vital capacities; yet failed to show a midposition change of even 10 per cent.

SUMMARY

The effects of pneumoperitoneum on diaphragm position, lung volumes, nitrogen elimination during oxygen breathing, and ventilation-perfusion relationships were measured in young men with pulmonary tuberculosis who were studied in the supine position.

In most of 18 patients, pneumoperitoneum displaced the inspiratory, expiratory, and midpositions of the diaphragm's cephalad. In a group of 33 patients, the corresponding lung volumes showed the following mean falls following treatment: total lung volume, 10 per cent; residual volume, 21.6 per cent; functional residual volume, 20.4 per cent. The vital capacity was minimally reduced in most patients (group mean = 4.4 per cent).

In 31 patients who demonstrated a 15 per cent or greater decrease in functional residual volume after the establishment of an effective pneumoperitoneum, the concentration of nitrogen in alveolar gas at the end of a seven-minute period of oxygen breathing was usually lower than it had been before that treatment was instituted.

The relationship between alveolar ventilation and perfusion was not consistently affected in the 10 patients given pneumoperitoneum.

The findings in this study support the conclusion that an adequate pneumoperitoneum produces collapse without disturbing the ventilation-perfusion relationships in certain persons with pulmonary tuberculosis.

SUMARIO

Algunos Efectos del Neumoperitoneo sobre la Respiración en los Tuberculosos Pulmonares

En jóvenes que padecían de tuberculosis pulmonar y fueron estudiados en decúbito supino, midiéronse los efectos del neumoperitoneo sobre la posición del diafragma, el volumen pulmonar, la eliminación de nitrógeno durante la respiración de oxígeno y las relaciones de ventilación-perfusión.

En la mayor parte de los sujetos, el neumoperitoneo desplazó hacia arriba el diafragma en posiciones inspiratoria, expiratoria y media. En un grupo de 33 enfermos, los correspondientes volúmenes pulmonares revelaron las siguientes bajas medias a continuación del tratamiento: volumen pulmonar total, 10 por ciento; volumen residual, 21.6 por ciento; volumen residual funcional, 20.4 por ciento. La capacidad vital disminuyó mínimamente en la mayor parte de los enfermos (media para el grupo = 4.4 por ciento).

En 31 enfermos que mostraron una disminución de 15 por ciento o más del volumen residual funcional después de establecerse un neumoperitoneo eficaz, la concentración de nitrógeno en el gas alveolar al cabo de un período de siete minutos de respiración de oxígeno fué por lo general más baja que antes de iniciarse el tratamiento.

La relación entre la ventilación y la perfusión alveolares no se afectó constantemente en 10 enfermos tratados con neumoperitoneo.

Los hallazgos de este estudio apoyan la conclusión de que un neumoperitoneo adecuado produce colapso sin trastornar las relaciones de ventilación-perfusión en ciertos individuos que tienen tuberculosis pulmonar.

RESUME

Certains effets du pneumopéritoine sur la respiration chez des malades atteints de tuberculose pulmonaire

Les effets du pneumopéritoine sur la position du diaphragme, le volume du poumon, l'élimination de l'azote au cours de l'inhalation d'oxygène et les rapports ventilation-perfusion ont été déterminés chez des jeunes hommes atteints de tuberculose pulmonaire et étudiés en position de supination.

Chez la plupart de ces 18 malades, le pneumopéritoine déplaçait les positions inspiratoires, expiratoires et moyennes du "dôme" du diaphragme. Dans un groupe de 33 malades, les volumes du poumon correspondants montraient à la suite du traitement les chutes moyennes suivantes: volume total du poumon, 10%; volume résiduel, 21,6%; volume fonctionnel résiduel, 20,4%. La capacité vitale était très légèrement réduite dans la plupart des cas (moyenne du groupe = 4,4%).

Chez 31 malades présentant une réduction du volume résiduel fonctionnel de 15%, ou plus, après création d'un pneumopéritoine efficace, la concentration de l'azote dans l'air alvéolaire, après inhalation d'oxygène pendant une période de sept minutes, était habituellement plus basse qu'avant l'instauration du pneumopéritoine.

Chez 10 malades, porteurs d'un pneumopéritoine, le rapport entre la ventilation alvéolaire et la perfusion n'était pas affecté de façon constante.

Les résultats obtenus dans cette étude viennent confirmer la conclusion qu'un pneumopéritoine adéquat produit le collapsus sans altérer les rapports ventilation perfusion, chez certains individus atteints de tuberculose pulmonaire.

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Our gratitude is due a number of hospital corpsmen whose skill and industry account for much of the work of this study. Among these are the following: John Briggs, Robert Rauf, David Prior, Richard Monsees, and Kenneth Gorr.

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A CLINICAL AND BACTERIOLOGIC CORRELATION OF RESECTED PULMONARY TUBERCULOUS LESIONS[1, 2]

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INTRODUCTION

A correlation of the morphology and bacteriology of tuberculous lesions resected after chemotherapy has been the basis of a continuing study at this hospital since August, 1951. The study was originally stimulated by certain pathologic concepts and preliminary observations by Medlar, D'Esopo, and others (1–5). It is hoped that eventually the effects of the types and duration of chemotherapy, cavity closure, the discharge of tubercle bacilli and their drug-susceptibility, the stage of disease, and other factors upon the bacteriologic findings in the resected tissue may be clearly defined. In this way, more definitive criteria for the resection of tuberculous lesions after chemotherapy could presumably be established.

The criteria employed by D'Esopo and his group for uniformity of chemotherapeutic evaluation prior to surgery consisted of disappearance of tubercle bacilli from the sputum by microscopy and culture, stability of the lesion by roentgenography, and closure of cavities. These criteria were termed by them "the therapeutic target point" (6). The majority of the patients in their series, which was highly selected by these criteria, had received no tuberculosis chemotherapy except for that received continuously prior to resection.

Not all of the present cases met these rigid clinical criteria at the time of surgery. It was found by the present writers and other investigators that cavity closure which was not evident after six months of chemotherapy was frequently associated with a rapidly increasing incidence of streptomycin-resistant strains of tubercle bacilli. This was the fact even when streptomycin had been used with other antituberculous drugs. The occurrence of this phenomenon offset any advantage from continued treatment directed toward cavity closure. As a consequence, preoperative chemotherapy in cavitary lesions was usually not pursued to the point of cavity closure if closure did not occur within the first six to nine months of therapy.

MATERIALS AND METHODS

Resections: The series reviewed represents 277 consecutive resections at this hospital in the two-year period from August 1, 1951, to August 1, 1953. Twenty-seven resections were dropped from the series due to failure to obtain adequate bacteriologic study of re-

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sected tissue or proof of tuberculous etiology. The net series for this report thus consists of 250 resections from 201 patients. There were 3 pneumonectomies, 46 lobectomies, and 201 resections of units smaller than a lobe, e. g., segmental or wedge resections or nodulectomy.

All but 2 patients were males. There were 9 nonwhites in the entire group. The distribution was from eighteen to sixty-five years, with the majority of patients in the third and fourth decades. The largest single group was in the third decade of life.

Cultural techniques: The resected specimens were divided under sterile precautions, and representative portions were given to the bacteriologist and pathologist for study. The material was received at the bacteriology laboratory in a sterile Petri dish and cut into small bits with scalpel and scissors under sterile precautions. The tissue was then homogenized in a motor-driven tissue grinder (Teslon Pestle) for five minutes.

The bacteriologic studies upon this homogenized material included: gram stains and Ziehl-Neelsen stain of both undigested and digested (3 per cent sodium hydroxide) portions; aerobic and anaerobic cultures on enriched agar; special cultures for *Actinomyces* and cultures on Sabouraud medium; and 4 cultures each of the digested and undigested portions on Löwenstein solid medium. Cultures were reported at the end of eight weeks, but were incubated for one year if negative at eight weeks.

Guinea pig inoculation: Digested and undigested portions of the specimens were inoculated into guinea pigs. Intradermal inoculations of Old Tuberculin (0.1 cc. of 1:200 dilution) were made in the guinea pigs before and four weeks after inoculation. Positive reactors (more than 1 cm. of induration) were sacrificed at four weeks, and nonreactors at eight weeks, after inoculation. The regional lymph nodes, livers, and spleens of the animals were studied by Ziehl-Neelsen stained smears and visual inspection for lesions. When no gross lesions of the liver or spleen were found, these organs were fixed in formalin for microscopic sections (H and E stain). If only the regional lymph nodes were involved, the nodes were inoculated into another guinea pig and planted on Löwenstein medium to provide more tubercle bacilli for further virulence tests.

Staining of tissue sections: Acid-fast staining of tissue sections was done routinely by the pathologist. Careful gross and microscopic study of the tissue was made to determine the presence or absence of cavitary lesions and bronchocavitary communications. The term "positive findings" used in this study refers to the demonstration of the presence of living tubercle bacilli by either culture or guinea pig inoculation, or both, in the tissue supplied to the bacteriologist.

RESULTS

Extent of disease: The distribution of cases according to duration of preoperative chemotherapy and extent of disease is shown in tables 1 and 2. Among the 201 patients, 16 per cent had minimal disease, 46 per cent had moderately advanced disease, and 38 per cent had far advanced disease. The extent of disease indicated is that existing at the start of chemotherapy. The duration of preoperative chemotherapy appeared to be related primarily to the extent of disease at the beginning of chemotherapy, and the percentage of positive findings was similarly related. A comparison of the findings according to the stage of disease for all durations of chemotherapy indicated that 33 per cent of the demonstrations of tubercle bacilli were in the lesions from the minimal group, 21 per cent from the moderately advanced lesions, and 34 per cent from the lesions in the far advanced group. None of the patients with minimal disease had received more than 8 months of preoperative chemotherapy.

Positive bacteriologic findings were obtained in 30 per cent of all resectioned specimens from patients treated with 8 months or less of chemotherapy, compared

TABLE 1

POSITIVE CULTURES FOR M. TUBERCULOSIS AMONG PULMONARY RESECTIONS IN 201 CONSECUTIVELY TREATED PATIENTS, CLASSIFIED BY DURATION OF PREOPERATIVE CHEMOTHERAPY

August 1, 1951–August 1, 1953

Duration of Preoperative Chemotherapy	Total Resections	Resected Specimens Containing Culturable Tubercle Bacilli			
		Number	Cavitary	Non-cavitary	Per Cent of Total
Total	250	70	42	28	28
Less than 5 months	86	26	16	10	30
Five to 8 months	90	27	16	11	30
Nine to 12 months	49	8	4	4	16
Thirteen months and over	25	9	6	3	36

TABLE 2

CULTURABLE TUBERCLE BACILLI AMONG RESECTIONS IN 201 CONSECUTIVELY TREATED PATIENTS AFTER SPECIFIED PERIODS OF PREOPERATIVE CHEMOTHERAPY, CLASSIFIED BY STAGE OF DISEASE

August 1, 1951–August 1, 1953

Stage of Disease	Total Resections	Resections Done After 8 Months of Treatment or Less					Resections Done After More Than 8 Months of Treatment				
		Total	With Culturable Tubercle Bacilli				Total	With Culturable Tubercle Bacilli			
			Number	Cavitary	Non-cavitary	Per Cent of Total		Number	Cavitary	Non-cavitary	Per Cent of Total
All stages	250	176	53	32	21	30	74	17	10	7	23
Minimal	39	39	13	5	8	33	0	0	0	0	0
Moderately advanced	116	83	20	12	8	24	33	5	1	4	15
Far advanced	95	54	20	15	5	37	41	12	9	3	29

with 23 per cent of positive findings in those treated for more than 8 months. Most of the findings of viable tubercle bacilli in the longer treatment group were from patients with far advanced tuberculosis. A comparison of the differences among the groups based upon extent of disease revealed that, in the group which received 8 months or less of therapy, only the difference between the moderately advanced and far advanced lesions was statistically significant ($P = <0.01$). There were no significant differences in the comparisons of moderately advanced disease treated more or less than 8 months with far advanced disease in a comparable category.

Duration and course of preoperative chemotherapy: The entire experience with all durations and courses of preoperative chemotherapy is presented in table 2. One hundred and seventy-six (70 per cent) of all resections and 53 (75 per cent) of all positive findings were in the group which received 8 months or less of chemotherapy. This includes all patients who received more than one course of chemotherapy if the particular course preceding surgery was less than 8 months. A

comparison with the results in the group treated for more than 8 months suggests that the shorter duration of preoperative therapy in itself is a factor directly related to the incidence of positive findings. However, the group which received the shorter duration of therapy contained 75 per cent of the cases of cavitary disease demonstrable at surgery. This suggests that the higher incidence of positive findings was a factor of the extent and component of the disease process rather than the duration of therapy because cavitary cases were not uniformly treated to the "target point" in this series.

The effect of various antimicrobial regimens upon the bacteriologic findings in the resected tissue is shown in table 3. These apply only to patients who had received no tuberculosis chemotherapy except for that received continuously prior to resection, a group which received 68 per cent of all courses given. The regimen of streptomycin, 1 gm. daily, appeared to have been more effective than that of the twice weekly regimen, and the difference was statistically significant ($P = <0.02$). Comparison of the twice weekly regimen with the regimen of 0.5 gm. of streptomycin daily revealed no significant difference. A comparison of the differences between both daily regimens was significant ($P = <0.01$), however, and again indicated a therapeutic advantage for the 1 gm. daily regimen. No advantage of the 0.5 gm. daily regimen over the twice weekly regimen was suggested by this analysis.

The results with the isoniazid regimens appeared remarkably similar. The use

TABLE 3

CULTURES FOR M. TUBERCULOSIS OF TISSUE FROM 169 TUBERCULOUS LESIONS RESECTED FROM PATIENTS WHO RECEIVED A SINGLE CONTINUOUS COURSE OF PREOPERATIVE CHEMOTHERAPY

Preoperative Course	Streptomycin 1 Gm./Day PAS 12 Gm./Day		Streptomycin 1 Gm. 2× Week PAS 12 Gm./Day		Streptomycin 0.5 Gm./Day PAS 12 Gm./Day		Isoniazid Daily 150–300 Mg.		Streptomycin 1 Gm. 2× Week Isoniazid Daily		Isoniazid 300 Mg. Daily PAS 12 Gm./Day		Total—All Regimens		
	Number	Positive*	Number	Positive	Number	Positive	Number	Positive	Number	Positive	Number	Positive	Number	Positive	Per Cent
Months															
0–4	2	0	25	7	8	5	5	3	6	2	3	2	49	19	39
5–8	10	2	35	11	5	1	9	3	7	4	2	0	68	21	31
9–12	4	0	28	6	2	0	1	0	2	1	0	0	37	7	19
13 plus	2	0	13	6	0	0	0	0	0	0	0	0	15	6	40
Total	18	2	101	30	15	6	15	6	15	7	5	2	169	53	31
Comparison with 81 resected lesions receiving re-treatment courses															
Total	9	0	41	11	6	0	3	0	16	4	7	2	81	17	21
													250	70	28

* Positive for tubercle bacilli by culture or guinea pig inoculation, or both.

of isoniazid alone has been discontinued on this service because of the relatively early loss of bacterial susceptibility to this drug.

The interpretation of the findings in which streptomycin–para-aminosalicylic acid (PAS) regimens were compared (table 3) was partially obscured because assignment to the various regimens had not been made by chance. Hence, it is possible that the observed differences were related more to the type of case treated than to differences among the regimens. Nevertheless, a similar analysis was made of 79 cases randomized among the streptomycin-PAS regimens. This separate analysis indicated that the results presented in table 3 are entirely comparable with those of the total group, with the same degree of statistical significance with respect to frequency of positive findings as in the non-randomized cases.

The percentage of positive bacteriologic findings in the streptomycin twice weekly regimen was equal (30 per cent) in each division which was composed of patients who had had treatment for 8 months or longer. The other regimens were used in too few cases for a valid comparison on this basis. A comparison of all cases, both previously treated and re-treated, by a similar division of duration of therapy indicates that positive bacteriologic findings were obtained in 30 per cent of the specimens resected from 176 patients who were treated for 8 months or less. In 74 resected specimens obtained after more than 8 months of therapy, the bacteriologic findings were positive in 23 per cent. These differences are not statistically significant, and the data again suggest that duration of therapy alone is not a significant factor in the recovery of tubercle bacilli from resected tissue.

Preoperative sputum status with respect to M. tuberculosis: The effect of preoperative sputum status with respect to tubercle bacilli and the presence or absence of cavitary disease at the time of resection upon the bacteriologic findings in the resected tissue are shown in figure 1. The per cent of positive bacteriologic findings was greatest in either cavitary or noncavitary disease when the sputum remained positive for tubercle bacilli by microscopy or culture at the time of surgery. In the present study, the sputum was considered to be negative for tubercle bacilli at surgery only when sputum or gastric cultures, or both, had been negative for at least 2 months prior to surgery. The percentage differences in the isolation of tubercle bacilli from the resected specimens of sputum-"positive" or -"negative" cavitary and noncavitary cases were statistically significant ($P = <0.01$). The differences between "positive" sputum cavitary and noncavitary cases and "negative" sputum cavitary and noncavitary cases were also significant to the same degree ($P = <0.01$).

The results were entirely comparable whether analysis was made of those patients treated for 8 months or less or those who received all durations of preoperative chemotherapy. The presence of cavitary disease in the resected tissue was the major factor in determining the percentage of positive bacteriologic findings. This percentage was significantly greater when the sputum remained positive for tubercle bacilli until the time of surgery.

Cavitary and noncavitary disease: There were 114 lesions in which cavity had not been observed by roentgenogram prior to and during chemotherapy or at

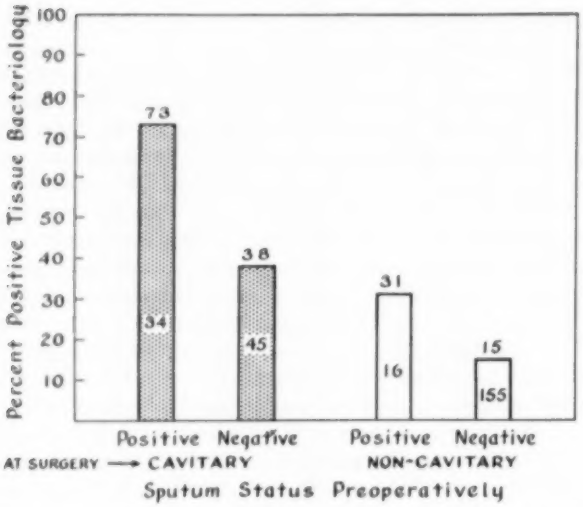

FIG. 1. Effect of preoperative sputum status upon positive bacteriologic findings in the resected tissue. Treatment with all durations of chemotherapy.

surgery. Tubercle bacilli were cultured from 21 (18 per cent) of these lesions; 5 of the 21 were from patients who had tubercle bacilli in the sputum at the time of surgery. The average duration of chemotherapy for the immediate preoperative course in those with positive bacteriologic findings was 6.4 months and totaled 7.0 months if prior therapy (re-treatment) was considered.

Fifty-seven cavitary lesions were observed prior to therapy which were subsequently lost to view by roentgenogram and were not found in the resected tissue. Tubercle bacilli were cultured from 7 (12 per cent) of these lesions, 2 of which were from patients who were still discharging tubercle bacilli at the time of surgery. The average duration of chemotherapy in this group of patients was 7.4 months for the immediate preoperative course and 8 months for total therapy.

There were 68 lesions in which cavity persisted by roentgenography throughout chemotherapy and was found at surgery. Thirty-seven (54 per cent) of these lesions yielded tubercle bacilli on culture, and 26 of the 37 were from patients whose sputum remained positive for tubercle bacilli until surgery. The average duration of the preoperative course of chemotherapy in this group was 6.5 months, with a value of 8 months for total therapy. All of the previously untreated patients who received a course of chemotherapy were found to have sputum negative for tubercle bacilli at the time of surgery. The 26 patients who continued to discharge bacilli had received more than one course of chemotherapy, with an average total therapy of 11 months.

In 11 instances no cavity was demonstrated prior to or during therapy; yet a cavity was found at surgery. Five (45 per cent) of these lesions yielded positive bacteriologic findings, but only one patient had tubercle bacilli in the sputum at

the time of surgery. The average duration of the immediate preoperative course of chemotherapy was 4.0 months, and the total therapy was 4.5 months.

Distribution of bacteriologic findings: The distribution of the bacteriologic findings in the resected lesions is shown in figure 2. Two hundred and one specimens (80 per cent) were positive for acid-fast bacilli on microscopy among the 250 resections, but only 70 (28 per cent) of all of the tissues examined from these resections yielded *M. tuberculosis* on culture. Of these cultures, 42 (60 per cent) were from lesions which were cavitary at resection. The entire distribution of lesions in the series reveals that 32 per cent were cavitary and 68 per cent were noncavitary at resection. In 9 cases, tubercle bacilli were cultured from specimens which were negative on microscopy. There were 41 cases in which microscopy, culture, and guinea pig inoculation of the resected specimens were negative for tubercle bacilli. Four of this latter group were cavitary at surgery. The influence of the patent cavity upon positive bacteriologic findings should be emphasized, for two-thirds of all positive bacteriologic findings were obtained from only one-third of the cases.

Streptomycin susceptibility: The streptomycin susceptibility of the tubercle bacilli recovered from resected tissue was determined in 53 instances, the majority of the cultures having been derived from cavitary lesions. Drug resistance was defined as the ability of the strain of tubercle bacilli to grow in 10 γ of streptomycin per ml. Most of the resistant strains also showed growth in 100 γ of streptomycin. The division between drug susceptibility and drug resistance was quite sharp for all strains of tubercle bacilli which were tested.

Of a total of 32 cavitary lesions, 13 yielded drug-resistant tubercle bacilli, all but one of which were resected from patients whose sputum remained positive for tubercle bacilli until surgery. The average duration of the total period of

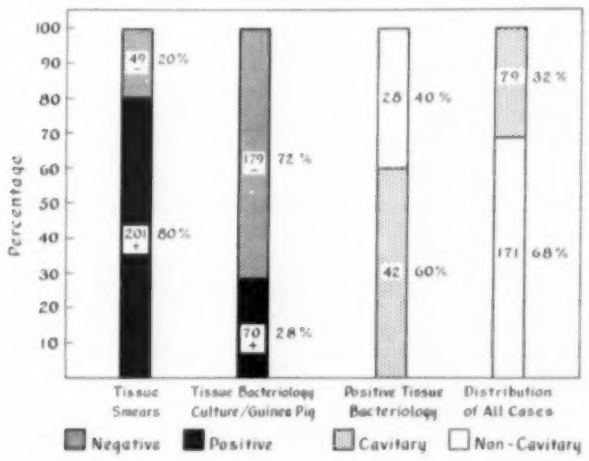

FIG. 2. Distribution of bacteriologic findings in resected tissue

chemotherapy prior to resection was 14 months. There were 19 cavitary lesions which yielded drug-susceptible tubercle bacilli; yet 12 of this group of patients had tubercle bacilli in the sputum until the time of surgery. The average duration of chemotherapy prior to resection in this group of patients was 6.3 months.

Similar studies were done on tubercle bacilli cultured from 21 noncavitary lesions. Four patients in this group continued to discharge tubercle bacilli until the time of surgery. Only 2 patient strains which were drug resistant were found after an average of 8 months of chemotherapy. The remaining 19 lesions in the group yielded drug-susceptible organisms, and they were obtained from patients who had received an average of 6.8 months of therapy prior to resection.

Only 5 of the entire group of patients in this phase of the study had tubercle bacilli which were streptomycin resistant prior to surgery both in the sputum and in the resected tissue. All of these had cavitary lesions and had received long courses of chemotherapy. All other patients had drug-susceptible tubercle bacilli in the sputum prior to surgery. The influence of a shorter length of preoperative chemotherapy upon the drug susceptibility of the tubercle bacilli was illustrated, particularly in the cavitary group. The advantage of resection of cavitary lesions from patients who continue to discharge tubercle bacilli despite six to eight months of chemotherapy confirms earlier impressions of the present writers of the rapid emergence of drug resistance after that period.

Complications: There were 56 postoperative complications, including 5 deaths, among 54 patients of the entire group who were treated surgically (table 4). Recurrent or persistent pneumothorax requiring catheter aspiration was the largest single group of complications and, together with the cases of postoperative atelectasis requiring bronchoscopic intervention, accounted for more than half of the

TABLE 4

SURGICAL COMPLICATIONS IN 277 CONSECUTIVE RESECTIONS IN 228 PATIENTS
August 1, 1951–August 1, 1953

Complication	Number
Wound infections	8
Pneumothorax requiring catheter aspiration	17
Atelectasis	10
Bronchopleural fistula (4 with empyema)	7
Hemothorax (4 requiring thoracotomy for control)	5
Empyema	1
Pulmonary infarction	1
Respiratory insufficiency with respiratory acidosis requiring tracheotomy	2
Deaths	5
Cardiac arrest during surgery	
Respiratory failure—acidosis	
Respiratory failure—pulmonary edema (2)	
Pulmonary embolism (massive)	
Total	56

total number of complications. The rate of surgical complications based upon the total of 277 resections was 20 per cent. This rate is identical with that for the net series of 250 resections, as is the distribution of complications. All deaths occurred in patients known to represent only fair to poor surgical risks and in whom more than a single lung segment was excised. The mortality rate based upon total resections was 1.8 per cent and based upon the number of patients was 2.1 per cent.

DISCUSSION

The initial concept of this and similar studies was based upon the resection of the residual necrotic lesion after adequate chemotherapy to remove a focus which might subsequently reactivate. Such lesions, particularly in the superior and posterior portions of the upper lobes, were considered to be potentially dangerous (2, 3), although the studies were made prior to the use of effective antituberculous drugs. The danger of the residual lesion has again been reflected in recent retrospective clinical studies of minimal tuberculous lesions, which were treated prior to chemotherapy, in which a relapse rate of 35 to 40 per cent has been reported (7, 8).

The finding that only a small percentage of resected tuberculous lesions treated with 6 to 8 months or more of chemotherapy prior to resection yielded tubercle bacilli on culture or guinea pig inoculation, despite the presence of acid-fast bacilli on microscopy, has spurred considerable investigation concerning the "viability" of those organisms. In the present series, 80 per cent of all tissue specimens were positive for acid-fast bacilli on microscopy; yet only 28 per cent of the specimens yielded culturable tubercle bacilli. Obviously, if the tubercle bacilli demonstrable only on microscopy were truly incapable of further vital processes, the necessity for resection in many cases might have been obviated.

The present study has led to the belief that resection is practicable and feasible earlier in the preoperative course of chemotherapy, although the total course should not be less than 12 months or not less than 6 months following resection. This is particularly applicable to patients with nodular and fibrocaseous disease, who frequently attain noninfectious sputum and stability by roentgenography after only a few months of chemotherapy. The residual lesions from such resections frequently show little tuberculous histologic activity, and viable tubercle bacilli are rarely found. Earlier discharge from the hospital is thus made possible by reducing the period of chemotherapy prior to surgery but not that of postoperative convalescence and chemotherapy.

Resection in the present series has been limited to single lesions of more than 0.5 cm. in diameter or contiguous lesions having a diameter of 1.0 cm. or more on the usual chest roentgenogram and planigrams. The inadequacy of all modern refinements of roentgenographic technique to demonstrate significant parenchymal disease palpable to the surgeon at thoracotomy has been a fairly frequent and disturbing finding. Although the criteria for resection may be revised in the future, it is unlikely that persistent cavitary lesions, destroyed lobes or

larger segments, bronchiectasis, and similar irreversible processes will escape surgical intervention.

The apparent therapeutic advantage of daily streptomycin (1 gm.) as compared with regimens of less frequent administration is of interest. Because of the rigid tests of the comparability of the cases treated on the three different streptomycin-PAS regimens, the earlier impression that the incidence of nonculturable tubercle bacilli is highest in lesions treated with the 1 gm. daily regimen is definitely substantiated. The further possibility that a longer course of streptomycin given twice weekly might later lead to a correspondingly low figure for culturable tubercle bacilli was not substantiated by the present data. When streptomycin was administered twice weekly for more than 8 months, the results were the same as in the patients who received the same regimen for 8 months or less.

SUMMARY

Acid-fast bacilli were demonstrated on microscopy from 80 per cent of 250 resected tuberculous pulmonary lesions, but only 28 per cent of these lesions yielded tubercle bacilli by the usual laboratory methods for culture and guinea pig inoculation.

The recovery of culturable tubercle bacilli appears to be most directly related to the persistence of cavitary lesions coupled with sputum positive for tubercle bacilli until the time of surgery.

The persistence of tubercle bacilli in the sputum until the time of surgery, even with noncavitary lesions, increases the incidence of the findings of culturable tubercle bacilli in the resected tissue.

The type and extent of disease at the beginning of chemotherapy are factors of greater significance in the recovery of culturable tubercle bacilli at the time of resection than is the duration of preoperative chemotherapy.

Tubercle bacilli cultured from resected lesions were uniformly resistant to streptomycin *in vitro* in cavitary lesions of patients who received more than an average of 8 months of preoperative chemotherapy.

The streptomycin susceptibility of tubercle bacilli cultured from resected lesions, cavitary or noncavitary, was retained when preoperative chemotherapy was given for less than an average of 8 months preoperatively.

The regimen of 1 gm. of streptomycin and 12 gm. of PAS daily appeared more effective than that of 1 gm. of streptomycin twice weekly and PAS daily in terms of recovery of culturable tubercle bacilli from the resected tissue.

SUMARIO

Correlación Clínico-Bacteriológica de las Lesiones de la Tuberculosis Pulmonar Resecadas

Obtuviéronse frotes bacterianos positivos de 80 por ciento de 250 lesiones tuberculosas resecadas, pero solamente 28 por ciento de dichas lesiones produjeron microbios viables con las habituales técnicas de cultivos y de inoculación de cobayos en el laboratorio.

La obtención de microbios viables parece estar enlazada más directamente con la persistencia de lesiones cavitarias, unido esto a un esputo positivo para bacilos tuberculosos hasta el momento de la intervención cruenta.

La persistencia de un esputo "positivo" hasta el momento de la operación, aun en lesiones no cavitarias, acrecienta la incidencia de hallazgos positivos (gérmenes viables) en el tejido resecado.

La forma y la extensión de la enfermedad al iniciar la quimioterapia son factores de mayor importancia que la duración de la quimioterapia preoperatoria en lo referente a la obtención de microbios viables al verificar la resección.

Los microbios cultivados de las lesiones resecadas se mostraron invariablemente resistentes a la estreptomicina, tratándose de lesiones cavitarias en enfermos que recibieron por término medio más de ocho meses de quimioterapia preoperatoria.

Retuvieron su susceptibilidad a la estreptomicina los microbios cultivados de lesiones resecadas, ya cavitarias o no, cuando se administró quimioterapia preoperatoriamente menos de un promedio de ocho meses.

El régimen que emplea diariamente 1 gm. de estreptomicina y 12 gms. de PAS pareció ser más eficaz que el de 1 gm. de estreptomicina dos veces semanales y PAS diariamente, a base de la obtención de gérmenes viables en los tejidos resecados.

RESUME

Corrélation clinico-bactériologique des lésions réséquées dans la tuberculose pulmonaire

Des étalements bactériens positifs ont été obtenus dans 80% des lésions tuberculeuses réséquées sur 250 spéciments de résection, mais 28% seulement d'entre eux ont permis d'obtenir des bacilles viables par les méthodes de culture usuelles et l'inoculation au cobaye.

L'isolement d'organismes viables paraît dépendre principalement de la persistance de lésions cavitaires accompagnées d'expectoration positive pour le bacille tuberculeux au moment de l'intervention chirurgicale.

La persistance de crachats "positifs" au moment de l'intervention, même quand il s'agit de lésions non-cavitaires, accroît l'incidence des résultats positifs (bacilles viables) dans les tissus de résection.

La forme et l'étendue de l'affection au début de la chimiothérapie sont des facteurs beaucoup plus importants, en ce qui concerne l'isolement de bacilles viables au moment de la résection, que ne l'est la durée de la chimiothérapie préopératoire.

Les microorganismes cultivés à partir des lésions réséquées se sont montrés constamment résistants à la streptomycine dans le cas de lésions cavitaires provenant de malades soumis à la chimiothérapie préopératoire pendant une période dépassant la moyenne de huit mois.

La sensibilité à la streptomycine des microorganismes cultivés à partir de lésions réséquées, cavitaires ou non cavitaires, était conservée quand la période de chimiothérapie préopératoire était inférieure à une moyenne de huit mois avant la résection. La cure qui consiste à administrer 1 g de streptomycine et 12 g de PAS par jour s'est montrée plus efficace que l'administration de 1 g de streptomycine deux fois par semaine et de PAS une fois par jour, comme le prouve la possibilité de récupérer des bacilles viables à partir des tissus réséqués.

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THE INTERMITTENT USE OF STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE SULFATE IN THE THERAPY OF PULMONARY TUBERCULOSIS[1, 2, 3]

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Streptomycylidene isonicotinyl hydrazine sulfate[4] (SIH) is a molecular combination which contains the equivalent of 1 gm. of streptomycin base and 236 mg. of isoniazid. Following a single intramuscular injection of 1.4 gm. of the compound, it has been found that drug concentrations were produced in the blood comparable to those following the injection of 1 gm. of streptomycin sulfate. Satisfactory isoniazid concentrations in the blood following injection of the compound have also been reported (1). Hobby and Lenert have reported that streptomycylidene isonicotinyl hydrazine given intermittently produced demonstrable therapeutic effects in animals (2). This indicated the possibility of intermittent use in the treatment of humans, and it appeared advisable to test the effectiveness of this chemotherapy. The combined use of the two most potent antimicrobials effective against *Mycobacterium tuberculosis* has disadvantages for some patients when isoniazid is given daily. In some situations, the administration of oral antimicrobials presents practical problems difficult to overcome.

PLAN OF STUDY

Patients admitted to the Chronic Chest Disease Service who had had no prior therapy with streptomycin or isoniazid were selected for study. In the meantime, other patients previously treated with either drug who became eligible for drug therapy were assigned to the study because it was thought that the effectiveness of the new regimen should also be tested among previously treated patients. Unfortunately, an adequate control group could not be selected for these patients.

The patients who received the intermittent streptomycylidene isonicotinyl hydrazine regimen were given 1.4 gm. of drug twice weekly except when the temperature at the time of initial observation exceeded 101° F. These patients were given 1.4 gm. daily for ten days before being placed on the intermittent routine.

The control group was not selected at random, but was composed of patients who were receiving streptomycin sulfate, 1 gm. twice weekly, plus 3 to 6 mg. of isoniazid per kg.

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[1] From the Chronic Chest Disease Service of Howard University, Freedmen's Hospital, Washington, D. C.

[2] This study was supported by a grant from Chas. Pfizer & Co., Inc., Brooklyn, New York.

[3] Portions of this study were presented at the Thirteenth Conference on Chemotherapy in Tuberculosis, Veterans Administration, Army, and Navy, St. Louis, February, 10, 1954.

[4] The streptomycylidene isonicotinyl hydrazine sulfate used in this study was supplied as Streptohydrazid® by Dr. Gladys L. Hobby, of the Research Laboratories, Chas. Pfizer & Co., Inc., Brooklyn, New York.

and others who were receiving 10 gm. of para-aminosalicylic acid (PAS) daily and 3 to 6 mg. of isoniazid per kg. For this reason, comparisons involving the development of streptomycin-resistant strains of tubercle bacilli are impossible, and any conclusions on this subject must be approached with great caution.

All of the patients except those with minimal disease had sputum positive for acid-fast bacilli on microscopy. The results of cultures are reported only for the period just preceding the start of the study.

Bacteriologic observations were made monthly, but are reported at eight-week periods. In the compilation of bacteriologic data, the least favorable report of positivity or bacterial drug-resistance is used. This procedure has produced more consistent serial information on individual patients and it compensates for negative findings due to faulty technique in the collection, delivery, or laboratory handling of the specimens.

Chest roentgenograms were taken monthly. These were single, posterior-anterior projections. Lateral films were obtained in the observation months. These were read and compared with the initial film. Comparisons which are analyzed here are based upon a staff consensus which indicated for each film *significant improvement* over the first film, *no change*, or *significant worsening*. Cavities are recorded as present or apparently closed.

For all of the patients, prior trends could be established on the basis of a historical film, clinical history, and symptoms. This determination involves a certain amount of individual bias, but is included as the pretreatment data in the tabulation of roentgenographic changes.

Toxicity observations were based upon systematic interviews of patients by a nurse trained in this work, a resident physician who reviewed all complaints, and upon the daily reports of the nursing team which administered all antimicrobials. Routine hemograms were recorded for each eight-week period, and morning urine specimens were recorded for the same periods. These reports were compiled and analyzed for significant variations for both groups.

Neither routine liver function tests nor tests of audio-vestibular damage could be performed.

Despite the small numbers of patients who had not received previous chemotherapy, life-table analysis has been employed for both the bacteriologic and roentgenographic observations.

The detailed study of the significance of bacterial resistance was hampered by the small numbers involved.

Description of Patients Studied at the Start of Therapy

The distribution of patients in the various groups by extent of disease is shown in table 1. The comparative distribution of patients by dynamic status of disease at the start of therapy is shown in table 2. These tabulations show a greater proportion of *far advanced* and

TABLE 1

DESCRIPTION OF 74 STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE-TREATED PATIENTS AND 64 CONTROL PATIENTS BY EXTENT OF DISEASE

Stage of Disease	Intermittent SIH Group		Control Group	
	Previously Untreated	Prior Therapy	Previously Untreated	Prior Therapy
Far advanced	16	20	27	12
Moderately advanced	16	14	16	4
Minimal	4	4	5	0
Subtotals	36	38	48	16
Totals	74		64	

TABLE 2

IMPROVEMENT VS. WORSENING AT START OF THERAPY

Dynamic Status of Disease	Intermittent SIH Group Previously Untreated	Control Group Previously Untreated	Intermittent SIH Group, Prior Therapy	Control Group, Prior Therapy
Improving	13	6	13	5
Nonprogressive	3	8	15	7
Worsening	20	34	10	4
Totals	36	48	38	16

TABLE 3

DISTRIBUTION OF PATIENTS STUDIED BY DURATION OF PRIOR TREATMENT WITH STREPTOMYCIN AND/OR ISONIAZID

Duration of Prior Treatment	Intermittent SIH Group	Control Group
months		
0–½	36	48
1–9	14	9
10+	24	7
Totals	74	64

worsening lesions among the control patients who had not received previous chemotherapy. This bias must be kept in mind in the interpretation of comparative results.

The age and sex distribution of patients in the two groups was comparable.

The duration of prior treatment by assigned treatment regimens is shown in table 3. Unfortunately, the small number of these patients assigned to daily isoniazid regimens does not permit comparison with those who were assigned to the intermittent SIH regimen.

OBSERVATIONS

Evidences of Drug Toxicity

The intramuscular administration of streptomycylidene isonicotinyl hydrazine caused severe initial reactions in 4 patients who were consequently dropped from study and continued successfully on streptomycin twice weekly and daily isoniazid. The symptoms were malaise, fever above 101°F., weakness, tinnitus, nausea, and vertigo. These occurred within ten minutes to four hours after the drug was given. The symptoms of these patients recurred with a second dose and were not prevented by the prior administration of adequate doses of antihistamines. All had had previous therapy with streptomycin; none had received isoniazid.

A fifth patient had a temperature of 101°F. one hour after each of three single intramuscular injections of SIH. An oral antihistamine given one hour before the injection successfully averted fever and permitted continuation of the regimen. After three months of intermittent therapy, fever no longer appeared when the antihistamine was omitted. This patient had been handling streptomycin for four years prior to observation.

Two other patients were removed from therapy with intermittent SIH after the first and fifth months because of a severe generalized eczematoid rash and peripheral neuritis, respectively. Both had received therapy with streptomycin, but not with isoniazid.

More than half of the other previously treated patients who received intermittent SIH experienced toxic symptoms which were not sufficiently serious to justify discontinuance of therapy. Less than 10 per cent of the patients who had had no previous chemotherapy complained of untoward reactions to SIH.

The type of complaints which appeared among the intermittently treated SIH patients were those commonly reported following streptomycin administration. The occurrence of peripheral neuritis and generalized nervous tremor immediately after injection are exceptions. Two patients were observed with these complaints.

Among the control patients, one-third of the small previously treated group complained of toxic symptoms, and approximately 5 per cent of the previously untreated patients complained of such symptoms.

An analysis and comparison of the hemograms between the intermittent SIH and daily isoniazid groups revealed no consistent differences between the two groups. Eosinophilia was occasionally observed in both treated and control subjects, but was neither frequent nor excessive.

The morning urine specimens of the test group showed no greater frequency of albuminuria than that observed among patients receiving intermittent streptomycin. This was not found to be persistent in any patient studied.

Local irritation at the site of injection was not a serious problem following the injection of SIH diluted in 3.1 cc. of distilled water.

Deaths

The deaths in each group by treatment regimens are shown in table 4. None of these deaths seem to be attributable either to failure of the assigned drug regimen to control the tuberculous infection or to drug toxicity.

Bacteriologic Studies

The tabulation of sputum cultures by successive eight-week periods is shown in tables 5, 5A, and figure 1. This experience was studied statistically to determine the significance of differences in the frequency of obtaining cultures positive for tubercle bacilli. As shown in the subscript to table 5, the differences in the frequency of positive cultures were not significant between the previously untreated patients who received intermittent SIH and those who received drug combinations with daily isoniazid. The probability of the differences being significantly in favor of either regimen is approximately one in twenty in 95 per cent of all patients observed.

In life-table 5 and figure 1, which compare the bacteriologic results for all patients in the intermittent regimen group and all control patients, there is demonstrable statistical significance between the frequency of the finding of tubercle bacilli by culture in the sixteenth- and twenty-fourth-week comparisons.

TABLE 4

ANALYSIS OF DEATHS

Number of Patients	Age	Sex	Hospital Month	Extent of Disease	Duration of Therapy	Cause of Death
Original SIH Treated						
1	63	M	8	Far advanced	7 months	Pulmonary embolism
SIH Prior Therapy						
1	53	M	32	Far advanced Carcinoma	5 months	Metastatic carcinoma
2	38	M	43	Far advanced	7 months	Cardiac decompensation
Original Controls						
1	34	F	2	Far advanced	2 months	Surgical shock
Prior Therapy Controls						
1	58	F	25	Far advanced	5 months	Uremia

At sixteen weeks in the intermittently treated group, 27 per cent were discharging tubercle bacilli; in the daily isoniazid group, 12 per cent were discharging bacilli. At twenty-four weeks in the intermittent group, 28 per cent were similarly infectious; in the group receiving daily isoniazid, 16 per cent were infectious. Reference to table 3 shows a comparatively small proportion of previously treated patients in the daily isoniazid control patients and more previously treated patients among the intermittent SIH patients. The difference then may be assumed to be due to the difference in the composition of the two groups.

Drug-resistance of tubercle bacilli to 1 γ or more of isoniazid per ml. developed in the both previously untreated and in the control patients. No statistical analysis of this phenomenon is possible in a series of this size. The previously untreated patients who continue to discharge bacilli tend to discharge drug-resistant bacilli with greater frequency in the later months of treatment. This tendency does not, with the small number of later observations, differ significantly between the intermittently treated patients on SIH and those on daily isoniazid (table 5*A*). When the previously treated patients are reviewed as a whole, it is seen that they did not discharge isoniazid-resistant organisms with greater frequency than either the intermittently treated patients or those treated with daily isoniazid regimens (table 5*A*).

Two previously untreated patients observed at eight weeks developed and maintained strains of tubercle bacilli resistant to 30 γ or more of streptomycin for thirty-two weeks. This observation cannot be compared with the previously untreated control patients, since half had intermittent streptomycin and the others had daily PAS.

TABLE 5

RESULTS OF CULTURES FOR M. TUBERCULOSIS BY WEEKS AND REGIMENS

Weeks	Present at Start of Period		Completing Observation		No Examination		Died		Observed by Culture				Culture Positive				Growth 4+ in 1 γ or More of Isoniazid		Growth 4+ in 30 γ or More of Streptomycin		Total Positive Cultures Susceptible to Both			
									SIH		Controls		SIH		Controls						SIH		Controls	
	SIH	Controls	SIH	Controls	SIH	Controls	SIH	Controls	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	SIH	Controls	SIH	Controls	Number	Per Cent	Number	Per Cent
Previously Untreated Patients																								
Pretreatment	38	49	0	0	0	0	0	0	38	100	49	100	21	55	24	49	1	1	0	3	20	95	21	87
8 weeks	38	49	4	3	0	0	0	1	38	100	48	98	12	32	15	31	2	2	1	2	10	83	13	86
16 weeks	34	46	6	11	0	0	0	0	34	100	46	100	7	21	5	11	2	2	2	0	4	57	3	60
24 weeks	28	35	6	10	0	0	0	1	28	100	35	100	8	29	5	14	3	4	3	0	4	38	1	20
32 weeks	22	24	10	3	0	0	1	0	22	100	24	100	2	10	3	13	1	1	1	1	1	50	2	67
40 weeks	11	21	9	7	0	0	0	0	11	100	21	100	1	9	5	24	1	2	1	2	0	0	3	60
All Patients																								
Pretreatment	83	62	0	0	0	0	0	0	83	100	62	100	30	36	30	48	1	2	4	5	25	83	24	80
8 weeks	83	62	9	3	1	1	0	1	82	99	60	97	28	34	20	33	6	4	9	5	18	64	14	70
16 weeks	74	59	9	15	0	0	0	0	74	100	59	100	20	27	7	12	9	3	10	2	6	30	3	43
24 weeks	65	44	8	14	0	0	1	2	64	98	43	98	19	30	7	16	8	5	7	2	8	42	1	14
32 weeks	56	28	42	4	0	0	2	0	55	98	28	100	9	16	4	14	4	2	5	2	3	30	2	50
40 weeks	12	24	9	9	0	0	0	0	12	100	24	100	1	8	5	21	0	2	1	2	0	0	3	60

Subscript: The group of 38 previously untreated patients treated with SIH and the 49 control patients were examined bacteriologically at regular two-month intervals throughout the period of observation. Those patients in the two groups who remained under observation were compared at these same intervals to determine if there were any statistically significant differences in the proportions in each group showing cultures positive for tubercle bacilli for like time periods. Using the chi-square test with P at the 0.05 level, there were no significant differences found for these two groups of patients. However, a similar comparison of all patients treated with SIH with all patients treated with daily isoniazid regimens did yield significant differences in percentages of cultures positive for tubercle bacilli at the sixteen-week and twenty-four-week periods in favor of the daily isoniazid regimens. These, too, were tested according to chi-square, with P at the 0.05 level.

TABLE 5*A*

RESULTS OF CULTURES FOR M. TUBERCULOSIS BY WEEKS SHOWING TOTAL POSITIVE CULTURES, NUMBER AND PER CENT RESISTANT TO DRUG REGIMEN

Weeks	Total Positive Cultures for SIH Treated and Control Patients	Total Resistant to 1γ or More of Isoniazid	Total Positive Cultures for Intermittent SIH Patients	Total Resistant to Isoniazid on Intermittent SIH Regimen, 1 γ or More	Total Positive Cultures in Control Patients	Total Control Patients Resistant to 1 γ or More of Isoniazid
Previously Untreated Patients						
		per cent		*per cent*		*per cent*
8	27	4–15	12	2–17	15	2–13
16	12	4–33	7	2–29	5	2–40
24	12	7–58	8	3–38	4	4–100
32	5	2–40	2	1–50	3	1–33
40	6	3–50	1	1–100	5	2–40
All Patients						
8	48	10–21	28	6–21	20	4–20
16	27	12–44	20	9–45	7	3–43
24	26	13–50	19	8–42	7	5–71
32	13	6–46	9	4–44	4	2–50
40	6	2–33	1	0–0	5	2–40

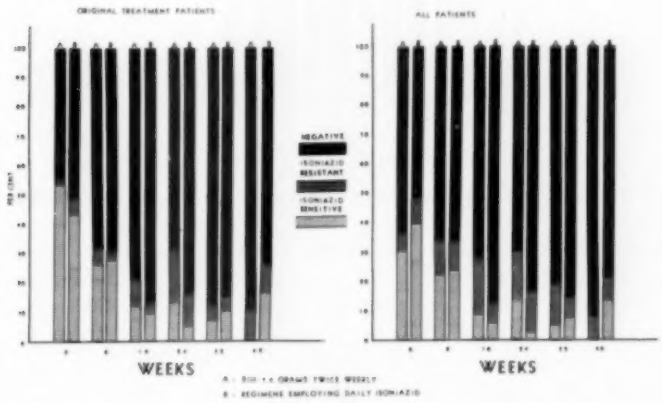

FIG. 1. Results of cultures for *M. tuberculosis* by weeks and regimens

Changes in the Chest Roentgenogram

The improvement in the chest roentgenogram compared for the various regimens is shown in figures 2 and 2*A* and table 6.

The intermittent regimens in the previously untreated and control patients showed evidence of improvement by roentgenography which is statistically comparable with that observed in the controls (*see* subscript to table 6). Sig-

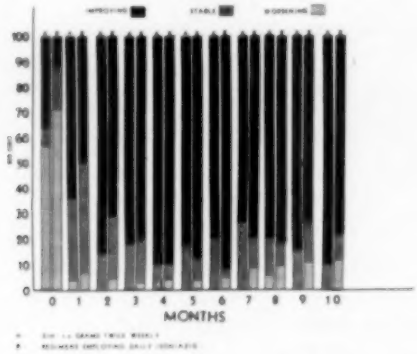

FIG. 2. Chest roentgenographic changes by months of observation in previously untreated patients.

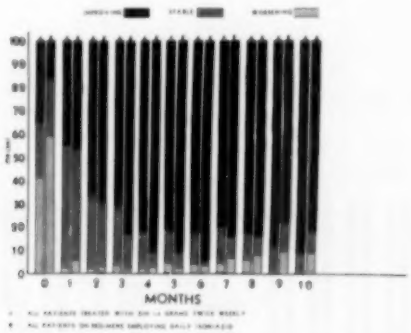

FIG. 2A. Chest roentgenographic changes by months of observation in all patients.

nificant differences between the previously untreated patients occurred in the column entitled "Apparent Cavity Closure" in favor of the intermittent group. Twenty-four per cent of those on intermittent SIH observed for cavity showed apparent closure at two months, as compared with 3 per cent of the control patients. At ten months, 89 per cent of the patients on intermittent SIH showed cavity closure, as compared with 53 per cent of the control patients. This can be interpreted as possibly due to the fact that approximately 8 of the original intermittent SIH patients had ten consecutive initial daily injections of SIH.

Other Observations

The relative effect of the two regimens upon fever, cough, and expectoration was not studied. Weight change in terms of per cent of starting weight gained per month of observation averaged 9.6 percentage parts per month for 72 intermittent SIH patients and a mean of 8.2 percentage parts per month for 54 daily isoniazid patients. This difference was not striking.

TABLE 6

Chest Roentgenographic Changes by Months and Regimens

Duration of Treatment, Months	Present at Start of Period		Completing Observation		Died		Number Observed		Significant Improvement				No Change				Worsening				Worsening at Start of Treatment				Observed for Cavity				Apparent Cavity Closure			
									SIH		Controls		SIH		Controls		SIH		Controls		SIH		Controls		SIH		Controls		SIH		Controls	
	SIH	Controls	SIH	Controls	SIH	Controls	SIH	Controls	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Previously Untreated Patients																																
1	36	48	0	0	0	0	36	48	23	64	24	50	12	33	21	44	1	3	3	6	20	56	34	71	30	83	39	81	0	0	0	0
2	36	48	1	1	0	0	35	48	30	86	34	71	5	14	12	25	0	0	2	4	18	51	34	71	29	83	39	81	7	24	1	3
3	35	47	3	5	0	0	34	47	28	82	40	85	6	18	6	13	0	0	1	2	20	59	34	72	29	85	34	72	6	21	9	26
4	31	42	1	8	0	0	31	42	28	90	38	90	3	10	3	7	0	0	1	3	18	58	31	74	27	87	35	83	10	37	15	43
5	30	34	2	7	0	0	29	33	24	83	29	88	3	17	3	9	0	0	1	3	17	59	24	73	25	86	30	91	9	36	13	43
6	27	27	3	2	0	0	25	27	20	80	25	92	5	20	1	4	0	0	1	4	15	60	20	74	22	88	25	92	10	45	11	41
7	24	25	4	3	0	1	23	25	17	74	20	80	6	26	3	12	0	0	2	8	14	61	19	76	20	87	23	92	12	60	10	40
8	20	21	6	2	1	0	20	21	16	80	17	81	3	15	2	9	1	5	2	9	13	65	17	81	13	65	19	90	11	84	10	52
9	13	19	3	1	0	0	13	19	11	85	14	74	2	15	3	16	0	0	2	10	8	62	16	84	11	85	18	95	8	73	9	50
10	10	18	4	5	0	0	10	18	9	90	14	78	1	10	2	11	0	0	2	11	7	70	15	83	9	90	17	94	8	89	9	53
All Patients																																
1	74	64	0	0	0	0	74	64	33	45	31	48	39	53	30	37	2	2	3	5	30	41	38	59	56	76	52	81	0	0	0	0
2	74	64	3	1	0	0	73	64	49	67	45	70	23	32	17	27	1	1	2	3	29	40	38	59	54	74	52	81	8	15	2	4
3	71	63	7	8	0	0	69	63	49	71	52	83	18	26	10	16	2	3	1	1	28	41	38	60	53	77	47	75	7	13	10	21
4	63	55	1	11	0	0	62	54	51	82	49	91	10	16	4	7	1	2	1	2	25	40	35	65	46	74	45	83	13	28	16	36
5	62	44	3	8	1	1	59	42	48	81	38	91	9	15	3	7	2	4	1	2	23	39	27	64	43	73	38	90	13	30	15	39
6	57	35	5	4	0	0	54	35	45	83	33	94	7	13	1	3	2	4	1	3	21	39	22	63	40	74	32	91	14	35	12	38
7	52	31	5	5	0	1	50	31	40	80	26	84	8	16	3	10	2	4	2	6	19	38	21	68	37	74	28	90	16	43	11	39
8	47	25	23	2	1	0	47	25	39	83	21	84	5	11	2	8	3	6	2	8	18	38	18	72	28	60	22	88	16	57	10	45
9	17	23	6	1	0	0	17	23	15	88	18	78	2	12	3	13	0	0	2	9	10	59	17	74	13	76	21	91	9	69	9	43
10	11	22	4	6	0	0	11	22	10	91	18	82	1	9	2	9	0	0	2	9	8	73	16	73	9	82	20	90	8	89	9	45

Subscript: Previously untreated patients on intermittent SIH and patients who received daily isoniazid regimens were observed at monthly intervals for changes in roentgenographic status. For those patients under observation for cavity, a comparison of the two groups showed statistically significant differences in the percentages with apparent cavity closures at two months and again at ten months. When all patients treated with intermittent SIH were compared for roentgenographic change with all patients treated with daily isoniazid regimens, there were no significant differences found at any time interval among percentages with apparent cavity closures.

In both of the above instances, the groups were tested according to chi-square, with P at the 0.05 level.

TABLE 7

PREVIOUSLY UNTREATED PATIENTS REACHING ARREST OF DISEASE OR SURGERY BY MONTHS

	Number	Total Patient-Months	Mean
Previously untreated patients reaching arrest of disease without major surgery			
Intermittent SIH	16	123	8.7
Controls	22	180	8.2
Previously untreated patients reaching major surgery			
Intermittent SIH	16	123	7.9
Controls	12	88	6.5

A more complete analysis is not possible with regard to the total experience.

On intermittent SIH, 2 patients with draining tuberculous sinuses, 2 patients with laryngeal lesions, and 3 with endobronchial lesions experienced satisfactory healing and have maintained their improvement.

From this group also, four specimens from excised lungs have been available for histologic study. Comparison with sections from the lungs of patients on the daily isoniazid therapies reveals no consistent differences in favor of either group.

Thirty-eight previously treated patients on intermittent SIH were observed for a total of 332 patient-months, an average of 7.2 patient-months. Forty-eight original treatment patients on daily isoniazid were observed for 354 patient-months, an average of 7.3 months.

In table 7, the writers have attempted to show in part the comparative results of the two regimens. Patients reaching the status of "arrested" with surgery and patients reaching major surgery are tabulated by average patient-months of treatment required to reach these objectives.

DISCUSSION

Intermittent streptomycylidene isonicotinyl hydrazine therapy without any additional drugs on a daily basis appears to yield significantly similar bacteriologic, roentgenographic, and clinical evidences of improvement when compared with established regimens which employ daily isoniazid with either intermittent streptomycin or daily PAS. Evidences of improvement between two previously untreated groups were not significantly different in frequency. A comparison of evidences of improvement by regimens in all groups, including those which had had prior therapy, gave some indication that intermittent therapy probably was equally as effective as daily isoniazid, but this was not definitely proved.

The statistical analysis of both bacteriologic and roentgenographic changes indicated the probability that intermittent SIH alone was comparably as effective as daily isoniazid regimens in previously untreated patients. This statistical analysis of comparatively small samples, which were not selected on a paired

and randomized basis, is to be accepted with caution until larger numbers of patients can be studied under more rigidly controlled conditions.

An attempt was made to relate the occurrence of roentgenographic worsening to the finding of isoniazid and/or streptomycin resistance in 14 patients who received intermittent SIH alone. Of these, only 4 showed concomitant or subsequent clinical worsening or spread of disease. These patients were discharging organisms resistant to 1 γ or more of isoniazid and 30 γ or more of streptomycin per ml. Unfortunately, no comparisons can be made with the control group.

The differences between the regimens reported here in both the bacteriologic and roentgenographic results are probably related to differences in the characteristics of the two patient groups. Any advantage which may be inferred should favor slightly the daily treatment group since this was weighted more heavily with patients with progressive and far advanced disease.

Drug toxicity can be anticipated with SIH administration in a few previously untreated patients. Most of the symptoms are of slight severity and the drug is well tolerated. There is a suggestion that injected isoniazid may enhance allergic sensitivity to streptomycin. This should be considered in planning therapy for patients who have had significant amounts of previous streptomycin therapy.

The evidences of clinical effectiveness of intermittent SIH seemed comparable with those of the daily regimens, although the rate of improvement may have been somewhat slower.

The study of these regimens among larger groups under more rigid statistical planning is advisable before more sweeping conclusions can be drawn. It seems safe to employ intermittent SIH for the therapy of pulmonary tuberculosis. Intermittent SIH cannot be recommended for the primary management of miliary, meningeal, or acute pneumonic tuberculosis, although it offers attractive possibilities as a maintenance program.

In view of the high therapeutic value of isoniazid and streptomycin and the danger that bacillary strains resistant to both will develop from their use together, it seems more reasonable, when these two drugs are used together, to administer them intermittently with or without the addition of daily PAS.

In some geographic areas or in other circumstances, when oral medication is impossible or uncertain, intermittent SIH offers an attractive possibility for controlled administration of combined drug therapy.

SUMMARY

Intermittent streptomycylidene isonicotinyl hydrazine sulfate, 1.4 gm., given twice weekly has been tested for a period of more than a year for bacteriologic, roentgenographic, clinical, and toxic manifestations. Results have been compared with those in regimens employing daily isoniazid.

Although significant differences are reported in certain comparisons of both the bacteriologic and roentgenographic studies, these appear to be due to errors of apportionment in the selection of regimens.

It may be concluded that the intermittent use of streptomycylidene isonico-

tinyl hydrazine sulfate in the present study appears substantially equal in effectiveness to that of regimens employing daily isoniazid. For patients who have had previous courses of therapy with daily isoniazid, daily PAS, or intermittent streptomycin, the use of intermittent streptomycylidene isonicotinyl hydrazine sulfate appears effective but has not been adequately tested.

It is suggested that this procedure may have great usefulness when daily oral drugs cannot be given with assurance.

The intermittent administration of streptomycylidene isonicotinyl hydrazine sulfate is deserving of further and more comprehensive analysis on a larger scale.

SUMARIO

El Uso Intermitente de Sulfato de Estreptomiclideno-Isonicotinil-Hidracina en la Terapéutica de la Tuberculosis Pulmonar

La administración intermitente de sulfato de estreptomiclideno-isonicotinil-hidracina, 1.4 gm., dos veces semanales, fué ensayada durante un período de más de un año con respecto a manifestaciones bacteriológicas, roentgenográficas, clínicas y tóxicas. Los resultados han sido comparados con los obtenidos por regímenes que emplean la isoniacida a diario.

Aunque aparecen diferencias significativas en partes de los estudios tanto bacteriológicos como radiográficos, éstas parecen deberse a errores de prorrateo en la selección de los regímenes.

Cabe deducir que el uso intermitente del sulfato de estreptomiclideno-isonicotinil-hidracina en este estudio dado parece ser substancialmente igual en eficacia al de los regímenes que utilizan la isoniacida diariamente. Para los enfermos que han recibido antes series de terapéutica diaria con isoniacida, con PAS diariamente, o estreptomicina intermitentemente, el uso intermitente del sulfato de estreptomiclideno-isonicotinil-hidracina parece eficaz, pero no ha sido comprobado adecuadamente.

Sugiérese que este procedimiento puede ser de mucha utilidad cuando no cabe administrar con seguridad drogas por vía oral a diario.

La administración intermitente del sulfato de estreptomiclideno-isonicotinil-hidracina merece un análisis ulterior más detenido y en mayor escala.

RESUME

L'administration intermittente de sulfate de streptomyclidene isonicotinyl hydrazine dans le traitement de la tuberculose pulmonaire

L'administration discontinue de sulfate de streptomyclidene isonicotinyl hydrazine, à la dose de 1,4 g, deux fois par semaine, a été expérimentée pendant une période de plus d'une année du point de vue bactériologique, radiologique et clinique ainsi que de la toxicité. Les résultats ont été comparés avec ceux obtenus par le traitement quotidien avec l'isoniazide.

Bien que des différences significatives figurent dans des rapports concernant des études bactériologiques ou radiologiques, ces écarts paraissent imputables à des erreurs de répartition dans la sélection des régimes thérapeutiques.

On peut conclure que, dans cette étude particulière, l'usage intermittent de sulfate de streptomyclidene isonicotinyl hydrazine a une efficacité sensiblement égale à celle des traitements comportant l'administration quotidienne d'isoniazide. Chez les malades ayant été traites antérieurement par l'administration isoniazide chaque jour par le PAS chaque jour ou au traitement discontinu par la streptomycine, l'usage intermittent de sulfate de

streptomyclidene isonicotinyl hydrazine se montre efficace; toutefois, il n'a pas été expérimenté de facon adéquate.

L'article suggère que ce procédé thérapeutique peut avoir une grande utilité quand les médications orales ne peuvent être administrées chaque jour avec certitude.

L'administration discontinue de sulfate de streptomyclidene isonicotinyl hydrazine mérite d'être étudiée plus complètement et plus extensivement sur une plus grande échelle.

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EXPERIMENTAL TUBERCULOUS MENINGITIS IN GUINEA PIGS: RESULTS OF TREATMENT WITH ISONIAZID, IPRONIAZID, STREPTOMYCIN, AND ISONIAZID-STREPTOMYCIN[1, 2]

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INTRODUCTION

The production of experimental tuberculous meningitis in guinea pigs and the results of immediate chemotherapy with streptomycin, para-aminosalicylic acid (PAS), and streptomycin–PAS have been described previously (1, 2). Other workers have subsequently reported similar experiments (3–6). The effect of iproniazid therapy in this type of infection has also been mentioned in a prior communication from this laboratory (7), and that of isoniazid in a later paper by Levaditi and co-workers (8). It is the purpose of this report to present the results of experiments in which various treatment regimens utilizing isoniazid, iproniazid, streptomycin, or isoniazid–streptomycin were used. The various chemotherapies were begun at different intervals after intracerebral infection and continued for five and one-half to six months, after which the animals were kept under observation for a further seven and one-half months.

MATERIALS AND METHODS

Fifty-nine guinea pigs which weighed between 600 and 800 gm. were infected intracerebrally in the manner previously described (1). Briefly, approximately 0.001 mg. (dry weight) of an H37Rv culture of human tubercle bacilli was introduced directly into the brain through a drill hole in the skull by means of a tuberculin syringe and small needle, with the animal under light ether anesthesia. The microorganisms were harvested from a two-week surface culture on modified Proskauer and Beck synthetic fluid medium, ground in a mortar, and suspended in physiologic saline solution in such a concentration that the desired inoculum was contained in 0.05 ml. The guinea pigs were then divided into ten groups as follows: an untreated or control group of 6 animals; 19 to be started on treatment the day after infection, 6 with iproniazid, 6 with isoniazid, and 7 with isoniazid-streptomycin; 12 to be started on treatment eight days after infection, 6 with iproniazid and 6 with isoniazid; 18 to be started on treatment sixteen days after infection, 6 with iproniazid, 6 with isoniazid, and 6 with streptomycin; and a group of 4 animals to be started on treatment with isoniazid-streptomycin only after signs of brain damage had become evident.

All drugs[3] were given intramuscularly in the form of an aqueous solution once daily for five and one-half to six months. The dosage of each drug is indicated in the footnote to table 1. It was necessary to reduce the amount of iproniazid from 20 to 10 mg. per day because of several deaths from drug toxicity on the higher dosage. Although no toxic signs

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[1] From the Trudeau Laboratory of The Trudeau-Saranac Institute, Trudeau, New York.

[2] This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

[3] The iproniazid and isoniazid were supplied by Hoffmann-La Roche, Inc., Nutley, New Jersey, as Marsilid® and Rimifon®; the streptomycin was supplied by Chas. Pfizer & Co., Inc., Brooklyn, New York.

were noted with 20 mg. of isoniazid, it was also reduced to 10 mg. daily for the purpose of comparison with iproniazid. At autopsy, the brain and upper cervical spinal cord were removed intact and placed in 10 per cent formalin. Other organs preserved for microscopic section were the cervical lymph nodes, lungs, liver, and spleen.

RESULTS

Untreated animals began to show clinical evidence of disease on approximately the fifteenth day. The signs were similar to those previously described, and consisted of listlessness, ataxia, generalized weakness, paralysis of the hindquarters, and, finally, general paralysis. The control animals all died between the sixteenth and twenty-fourth days after infection.

The results of therapy are indicated in table 1. It may be seen that, in general, animals treated within eight days after infection with any of the drug regimens did well. Only 3 died during the first four weeks of treatment, and all 3 deaths

TABLE 1

RESULTS OF TREATMENT IN GUINEA PIGS INFECTED INTRACEREBRALLY WITH M. TUBERCULOSIS, STRAIN H37Rv; THERAPY DISCONTINUED SIX MONTHS AFTER INFECTION

Treatment	Treatment Begun, Days After Infection	Died Within Six Months After Infection		Died, Days After Therapy Discontinued
		Number/ Total in Group	Survival Time, Days After Infection	
Controls		6/6	16, 19, 19, 23, 23, 24	
Iproniazid	1	2/6	9, 25 (both of drug toxicity)	36, 43, 107, 230(K)*
Isoniazid	1	0/6		34, 34, 40, 40, 48, 60
Isoniazid—streptomycin	1	1/7	134 (unknown cause)	40, 40, 46, 46, 74, 139
Iproniazid	8	1/6	16 (drug toxicity)	47, 48, 53, 82, 83
Isoniazid	8	0/6		58, 61, 70, 74, 84, 103
Iproniazid	16	5/6	19, 28, 28, 31, 35	75
Isoniazid	16	2/6	18, 25	39, 64, 85, 85†
Streptomycin	16	2/6	33, 120	139, 159, 159, 230(K)
Isoniazid—streptomycin	18–21 (Animals sick)	4/4	23, 23, 23, 24	

* Killed to terminate experiment.

† Died of streptococcal pneumonia and empyema.

Dosages of drugs were as follows:

Iproniazid and isoniazid—administered intramuscularly once daily, 20 mg. until the twenty-seventh day after infection, 10 mg. thereafter.

Streptomycin—administered intramuscularly 20 mg. once daily.

Isoniazid-streptomycin—streptomycin, 10 mg., isoniazid, 20 mg., once daily until the twenty-seventh day after infection; 10 mg. of each once daily thereafter.

were due to toxicity of iproniazid in the higher dosage. The groups for which treatment was delayed sixteen days were not completely protected. Four of the 12 animals in the delayed isoniazid and streptomycin groups died during treatment. Five of the 6 animals on delayed iproniazid died early, but their deaths were attributed to a combination of drug toxicity and the underlying tuberculous disease.

When therapy was delayed until the animals showed definite signs of meningitis or brain damage, the isoniazid-streptomycin therapy was of no avail. All 4 animals died after two to five days of treatment, or twenty-three to twenty-four days after infection.

Six months after infection, all treatment regimens were discontinued and the surviving animals were observed for signs of relapse. The 36 guinea pigs living at this time all appeared healthy. Thirty-four days later the first of these animals died, and two hundred and thirty days after treatment was discontinued, there were only 2 animals surviving. They were killed to end the experiment. In the majority of cases, the deaths which followed post-treatment relapse were preceded by ten to fifteen days of weakness, ataxia, and, finally, progressive paralysis. A few animals exhibited merely a progressive generalized weakness without definite paralysis, and some showed only listlessness and refusal to eat for several days before death.

Comparison of Drug Regimens

No one of the four regimens (iproniazid, isoniazid, streptomycin, or isoniazid-streptomycin) showed any real superiority over the others. Comparison of tuberculin skin tests, survival time, amount of gross disease at autopsy, and histopathologic studies revealed no significant differences among groups of animals started on therapy at the same time.

Gross Autopsy Findings

The brains of the untreated animals exhibited varying amounts of thickening and grayish exudate at the base. The animal which died after sixteen days showed no gross disease in the spleen, liver, or lungs; the cervical nodes were enlarged but not caseous. The 5 animals which died between the nineteenth and twenty-fourth days all showed gross tubercles in the spleen, with enlarged and caseous cervical lymph nodes. The lungs and livers were free of gross disease.

The findings in the animals for which treatment was delayed until after signs of disease had appeared were similar to those of the control group.

In the group started on treatment one day after infection, no gross tuberculous lesions were seen in the 3 animals which died eight, twenty-four, and one hundred and thirty-three days later. The one guinea pig which died after eight days of treatment, begun eight days after infection, also was free of gross lesions.

In the sixteen-day delayed treatment group, the animal which died after three days of iproniazid showed engorgement of the superficial blood vessels of the brain and slight enlargement of the cervical nodes, but no disease elsewnere. Only slight enlargement of the cervical nodes was seen in the guinea pigs

which succumbed after two and eight days of isoniazid. The 4 animals which died after twelve to eighteen days of iproniazid therapy showed very little evidence of disease grossly, aside from slight enlargement of the cervical lymph nodes and a slight thickening at the base of the brain. Neither of the 2 animals which received streptomycin (sixteen and one hundred and three days) showed disease grossly.

The guinea pigs which succumbed after treatment had been discontinued generally showed an amount of gross tuberculous disease in the cervical nodes and abdominal organs in direct proportion to the survival time. Those which died within forty days usually had no gross lesions, whereas in those which survived longer the cervical nodes were enlarged and fibrocaseous and the spleens contained a few nodular tubercles. The more far advanced lesions were seen in the animals which survived longest. None showed lesions in the lungs or liver, nor could any changes be noted grossly on the brain surface. Of the 2 animals still alive seven and one-half months after treatment was discontinued, the one which had received streptomycin starting sixteen days after infection had gross tuberculous lesions in the cervical lymph nodes and spleen, whereas the animal which had been treated with iproniazid starting one day after infection revealed no evidence of gross disease.

Histopathologic Findings

Early deaths: The meninges in the control animals (figure 1*A* and *B*) were greatly thickened by an exudate consisting of densely packed lymphocytes and epithelioid cells. In many places this reaction could be seen extending into the brain substance around the walls of blood vessels. It contained foci of necrosis of various sizes in most animals. Early tuberculous reaction with necrosis could also be seen in the cervical lymph nodes; the spleens all showed microscopic evidence of tubercle formation, even in the animal which died sixteen days after infection. A similar picture was seen in those treated with isoniazid-streptomycin after signs of illness had appeared (figure 1*C*). No microscopic evidence of healing could be noted in the tuberculous tissue of these 4 guinea pigs, which died after two to five days of treatment.

Three animals in the group started on therapy one day after infection died during treatment; 2 after eight and twenty-four days of isoniazid, respectively, and one after one hundred and thirty-three days of isoniazid-streptomycin. None of these animals showed any evidence of disease in the brain or other organs. Only one animal died early in the group started on treatment eight days after infection; it succumbed after eight days of iproniazid and revealed only a moderate lymphocytic infiltration in the meninges and a hyperplastic cervical lymph node.

A different picture was seen in the groups for which treatment was delayed sixteen days. The 3 animals which died during the first nine days of treatment revealed essentially the same picture as the control animals. One of them (treated with isoniazid for two days) demonstrated three microscopic foci of lymphocytes and epithelioid cells close to the ependymal lining of the lateral

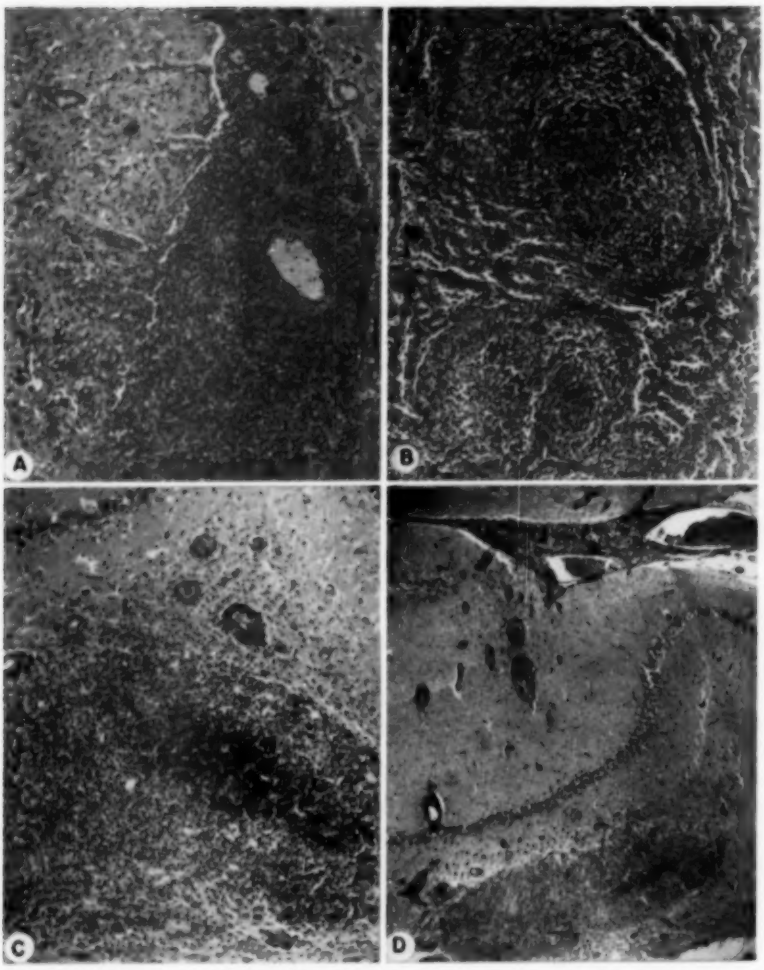

FIG. 1. *A*. Brain of control untreated guinea pig which died nineteen days after infection, showing pronounced lymphocytic and epithelioid cell reaction in the meninges extending into brain substance around the blood vessels. (100×) *B*. Spleen of same animal, showing early tubercle formation within Malpighian bodies. (100×) *C*. Brain of animal which died after five days of isoniazid-streptomycin begun after the onset of clinical illness. The intense meningeal inflammatory reaction is similar to that seen in the control guinea pig. (100×) *D*. Brain of animal which died after sixteen days of streptomycin, begun sixteen days after infection. A large active tubercle is in the brain substance at the bottom and meningeal inflammatory cell reaction is at the top. (50×)

ventricle, as illustrated in figure 3*A*. The 4 guinea pigs which died after twelve to eighteen days of iproniazid all showed definite evidence of healing in the form of fibrosis in the meningeal exudate and organization of the tubercles in the cervical nodes (figure 2). The animal which died after sixteen days of streptomycin therapy still showed the presence of a dense exudate, consisting of epithelioid cells and lymphocytes in the meninges, and several active tubercles in the brain substance (figure 1*D*).

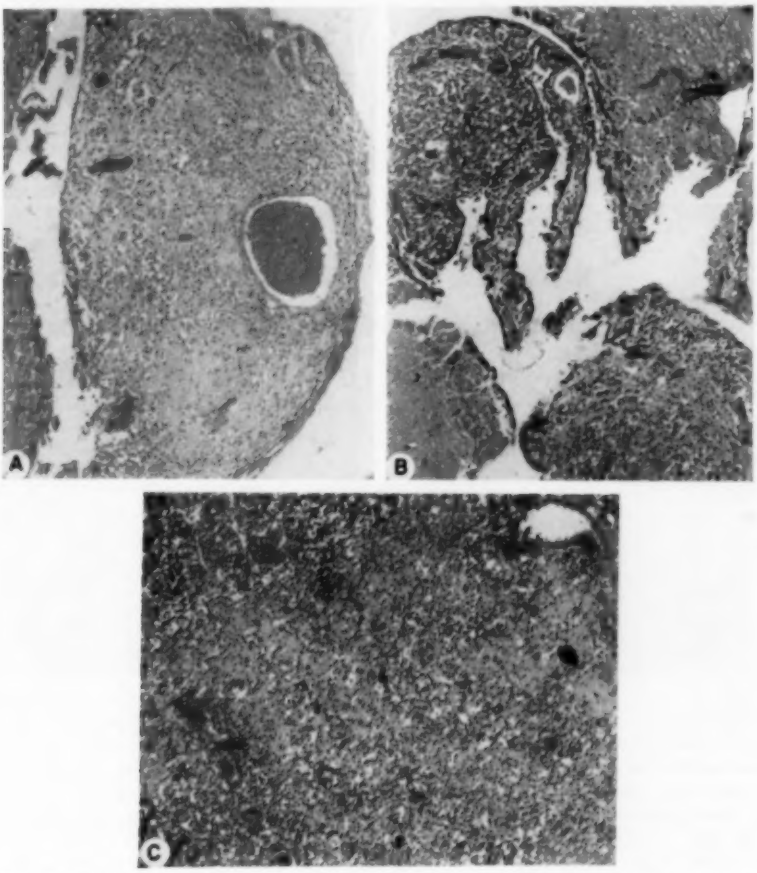

FIG. 2. These pictures illustrate the healing seen in the sixteen-day delayed treatment group after approximately two weeks of therapy. *A*. Brain of animal which died after fourteen days of iproniazid. There is an organizing tubercle in the meninges, relatively acellular because of almost complete replacement by collagen. (100×) *B*. Brain of animal which died after twelve days of iproniazid, showing organizing tubercles in the choroid plexus. (100×) *C*. Cervical node of same guinea pig, showing an organizing tubercle. The gray acellular material is collagen; the nuclei are those of lymphocytes and macrophages. (100×)

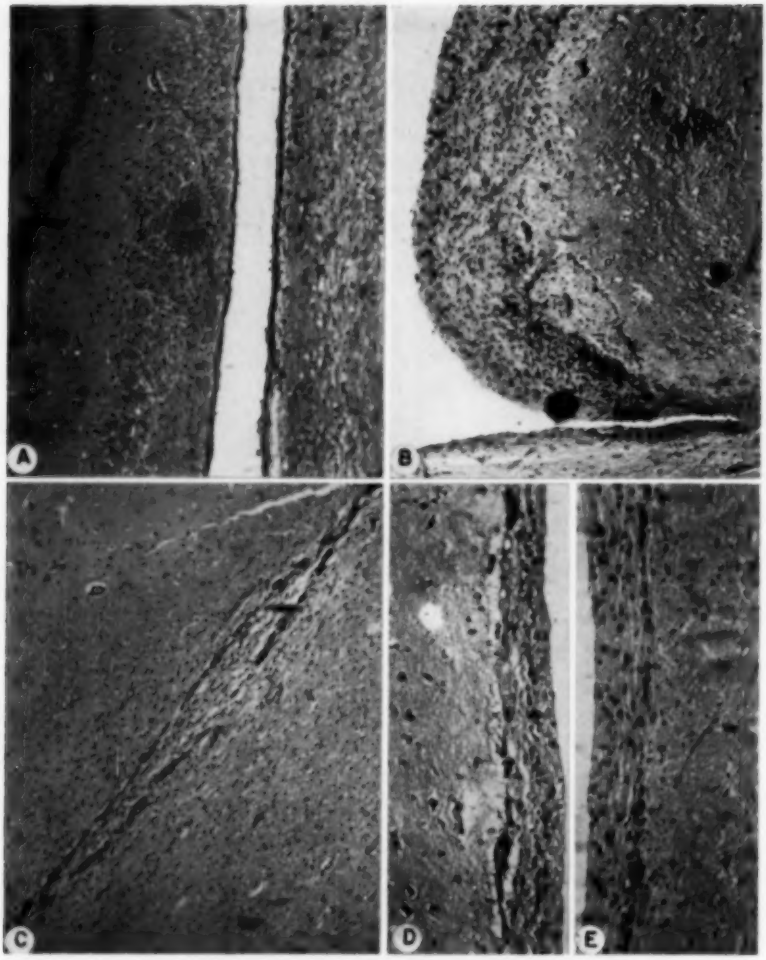

FIG. 3. These pictures illustrate the lesions seen in and around the lateral ventricles. *A*. Brain of animal which died after two days of isoniazid, begun sixteen days after infection, showing one of three small foci of lymphocytes and epithelioid cells around the lateral ventricle. A few acid-fast bacilli were seen in this lesion. Elsewhere, the meninges contained intense inflammatory reaction. The possible relationship between this lesion and the scars shown in *B* to *E* is discussed in the text. (100×) *B*. Brain of animal which died after one hundred three days of streptomycin, begun sixteen days after infection. There is a band of scar tissue lining the lateral ventricle; the nuclei are those of fibrocytes and lymphocytes. (100×) *C*. Brain of animal which died one hundred fifty-nine days after six months of streptomycin, begun sixteen days after infection, showing obliteration of the lumen of the lateral ventricle by organizing exudate. (100×) *D*. Brain of animal killed seven and one-half months after six months of streptomycin, begun sixteen days after infection. There is a band of scar tissue between the ependymal layer and the lumen of the lateral ventricle. (200×) *E*. Brain of animal which died one hundred fifty-nine days after six months of streptomycin, begun sixteen days after infection, showing a similar scar. The somewhat irregular row of large cells with dark nuclei is the ependyma. (200×)

Deaths after cessation of treatment: The meninges of these animals (figure 4*A*, *B*, and *C*) were thickened by tuberculous granulation tissue, consisting of epithelioid cells, lymphocytes, fibrosis, and a variable amount of tubercle formation with necrosis. In addition, many of them had active tubercle formation with necrosis in the brain substance, especially around the third ventricle. The amount of scar tissue in the meninges tended to be greater in the animals in which treatment was delayed. In 5 instances, a band of scar tissue was seen lying between the ependymal cells and the lumen of the lateral ventricles, and in one animal there was, in addition, an obliteration of part of the lumen of the ventricle by organized exudate (figure 3).

The cervical lymph nodes usually contained large epithelioid cell tubercles with a variable amount of necrosis. Microscopic tubercles were also seen in many of the spleens. The amount and severity of the disease in the cervical nodes and the spleen seemed to depend directly upon the length of survival after treatment had been stopped.

Of the 2 animals which survived to the end of the experiment, the one which had received iproniazid starting one day after infection revealed nothing abnormal except a very slight lymphocytic infiltration in the meninges. The other, which had been treated with streptomycin starting sixteen days after infection, had, in addition to gross lesions in the cervical nodes and the spleen, microscopic early tubercles in the lung, a very marked hydrocephalus (figure 4*D*), scarring in the lining of the third ventricle, and only very slight lymphocytic infiltration in the meninges.

Isolation of tubercle bacilli: Cultures for tubercle bacilli and drug-susceptibility tests were made from the grossly tuberculous spleens of 3 animals which had been treated for six months; the one in the streptomycin group which was killed seven and one-half months later, one treated with isoniazid-streptomycin which died forty-six days later, and one in the isoniazid group which died forty-eight days later. All three of the cultures were positive for tubercle bacilli, and the organisms were completely susceptible to streptomycin and isoniazid.

Hydrocephalus: It was noted in the previous report (2) that many of the animals which died after chemotherapy was discontinued exhibited a pronounced hydrocephalus. This observation was repeated in the present experiment. Dilatation of the ventricles was seen mainly in the animals which relapsed and died after treatment was discontinued, although it was seen to a slight extent in 3 animals of the sixteen-day delayed treatment group which died during therapy. A total of 19 guinea pigs exhibited some degree of hydrocephalus. In 9 of these animals, it was classified as moderate or marked in degree. There seemed to be no absolute relation between the hydrocephalus and the presence or absence of paralysis.

Tuberculin skin tests: Intradermal tests with 5 per cent Old Tuberculin were performed at frequent intervals. The results are presented in table 2.[4] It may be seen that, within fifteen days, the untreated animals showed positive reac-

[4] A further breakdown into individual treatment regimens is not given as there were no significant differences.

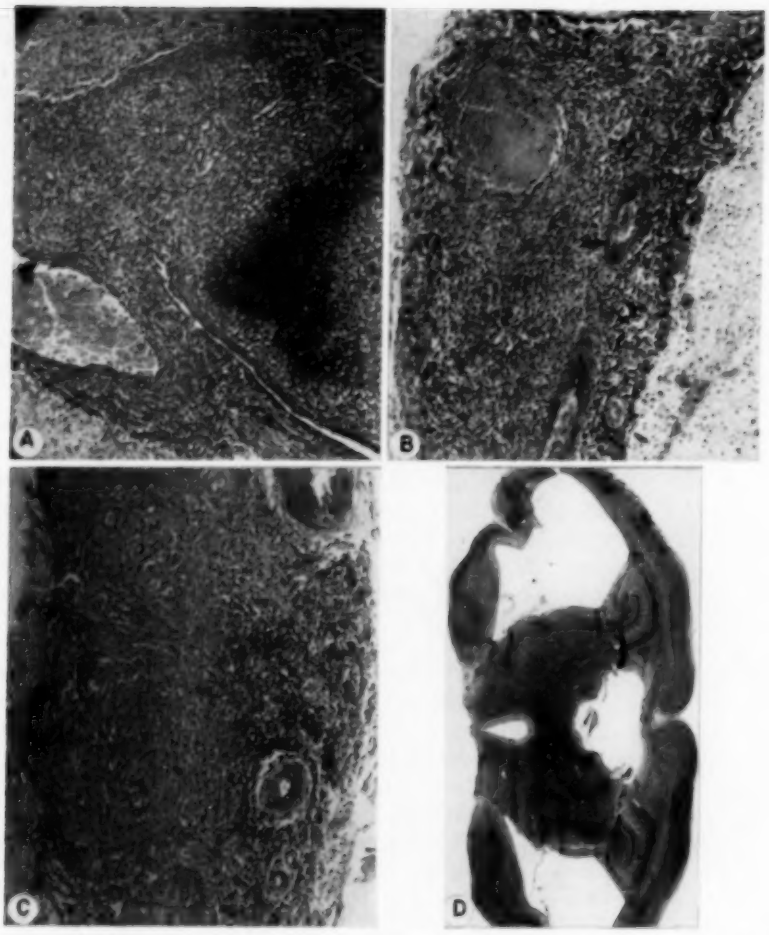

FIG. 4. Hydrocephalus and meningeal lesions in animals which died after drug therapy was discontinued. *A*. Brain of guinea pig which died thirty-six days after six months of iproniazid, begun one day after infection. The meninges are greatly thickened by an intense reaction composed of epithelioid cells with necrosis, a few lymphocytes, and much fibrous tissue. (100×) *B*. Brain of guinea pig which died seventy days after six months of isoniazid, begun eight days after infection. There is a marked meningeal reaction consisting of lymphocytes, epithelioid cells, and fibrosis. (100×) *C*. Brain of guinea pig which died one hundred fifty-nine days after six months of streptomycin, begun sixteen days after infection. There is a pronounced thickening of the basilar meninges due to scar tissue, collections of epithelioid cells, and foci of lymphocytic infiltration. (100×) *D*. Section through brain of animal killed seven and one-half months after six months of streptomycin, begun sixteen days after infection, showing pronounced hydrocephalus involving both lateral ventricles and the third ventricle. (3×)

TABLE 2

RESULTS OF INTRADERMAL TESTS WITH 5 PER CENT OLD TUBERCULIN

Time After Infection	Treatment Started, Days After Infection	Number of Animals Tested	Skin Test Results			
			Positive with Necrosis	Erythema and Induration Only	Doubtful	Negative
15 days	Untreated	22	8	14	0	0
	1	18	0	0	10	8
	8	12	0	10	2	0
27 days	1	17	0	0	5	12
	8	11	0	0	10	1
	16	13	5	7	0	1
41 days	16	10	6	4	0	0
4 months	1	17	0	1	5	11
	8	11	0	5	6	0
	16	10	2	5	3	0
7 months	1	9	0	4	3	2
	8	11	0	10	1	0
	16	8	2	0	6	0
9–11 months	1	1	0	0	0	1
	16	4	1	3	0	0

tions. The reactions in those treated one day after infection remained either negative or doubtful up to four months (with one exception at the four-month period). Most of the animals in the eight-day delayed treatment group showed tuberculin reactions which were weakly positive at fifteen days, doubtful or negative at twenty-seven days, and doubtful or weakly positive at four months. The majority in the sixteen-day delayed treatment group remained positive during therapy, although 3 of 10 showed only doubtful reactions at four months.

One month after therapy was discontinued, only 2 animals had strongly positive tuberculin reactions, 14 were weakly positive, and 12 were doubtful or negative. Later tests on surviving animals were all positive except in the one guinea pig treated with iproniazid which survived until the end of the experiment.

DISCUSSION

The tubercle bacilli inoculated into the brain obviously spread to other parts of the body quite rapidly, as evidenced by the fact that untreated animals showed evidence of disease in the cervical lymph nodes and the spleen sixteen to nineteen days after infection. Effective chemotherapy with isoniazid, iproniazid, or isoniazid-streptomycin begun one day after infection was able to prevent the appearance of a highly positive tuberculin skin test. This is in contrast with the findings of a previous experiment (1), in which 8 guinea pigs

treated with streptomycin alone (18 to 36 mg. daily) immediately after infection gave positive tuberculin tests on the fortieth day. Such early chemotherapy also prevented the appearance of detectable lesions in the brain and other organs as long as the treatment was continued, but as soon as it was stopped the tubercle bacilli, which must have remained viable during this period, were able to multiply and produce progressive tuberculous lesions. None of the drug regimens administered for six months, including isoniazid-streptomycin, was adequate to prevent subsequent relapse despite the fact that relatively large doses of each drug were used. The only guinea pig which had apparently been "cured" was the one treated with iproniazid and killed at the end of the experiment, in which the only possible evidence of disease was a very slight lymphocytic meningeal infiltration. All those which died after treatment had been discontinued showed evidence of relapsing tuberculous disease. It is possible that a longer period of treatment would give more lasting results.

The effect of delay of treatment was striking. There seemed to be a dividing line at approximately two weeks after infection, before which treatment was effective and after which it was of very little avail. Those animals not treated until they were obviously sick were beyond help by isoniazid-streptomycin therapy. It was interesting, however, that the guinea pigs in the delayed treatment group which survived the critical period shortly after the onset of therapy behaved similarly thereafter without regard to the length of time that had elapsed between infection and the start of treatment. Indeed, the group which survived the longest was that treated with streptomycin starting sixteen days after infection. Careful consideration of the available experimental evidence suggests that this difference was probably not significant.

In the sixteen-day delayed treatment group, healing in the form of organization and fibrosis was evident in the cervical lymph nodes and the meninges as early as twelve days after the onset of therapy, and 5 guinea pigs which died some time after treatment was stopped showed a band of fibrous tissue in the lining of the lateral ventricles between the ependymal layer and the lumen. The location of this scar suggests that the original exudate from which it was formed was present in the lumen of the lateral ventricles. In one instance, an actual obliteration of part of the lumen of the lateral ventricle by this organized exudate was seen (*see* figure 3*C*). Such a condition might account in part for the hydrocephalus seen in many of these animals. An alternative explanation for the scars in the lateral ventricle may be offered by the situation illustrated in figure 3*A*. The small foci of lymphocytes and epithelioid cells, which contained visible acid-fast bacilli, could conceivably develop into full-blown tubercles which would eventually rupture into the lumen of the lateral ventricle.

SUMMARY

Experimental tuberculous meningitis was produced in guinea pigs by intracerebral inoculation of a small number of the virulent human strain of *Mycobacterium tuberculosis* H37Rv. An attempt was then made to eradicate this infection by the administration of several different chemotherapy regimens

including iproniazid alone, isoniazid alone, streptomycin alone, and isoniazid-streptomycin. The effect of delay of therapy was studied by starting the drug either one, eight, or sixteen days after infection. A small group of animals was not treated until definite signs of illness were evident. All treatment was discontinued six months after infection, following which the surviving animals were observed for an additional seven and one-half months.

The results indicate that, in the dosage used, iproniazid, isoniazid, streptomycin, and isoniazid-streptomycin were able to control the experimental meningitis, provided the treatment was started within eight days after infection. With a delay of sixteen days, the protection offered by these drugs was not complete and, if treatment was delayed until the onset of definite illness, it was not beneficial. All of the untreated guinea pigs died sixteen to twenty-four days after infection.

All of the drug regimens used were of equal benefit in so far as could be determined by comparison of tuberculin skin tests, survival time, relapse rate, and tissue pathology.

None of the treatment regimens maintained for six months was sufficient to eradicate the tubercle bacilli completely, as all but 2 of the 36 animals which survived the entire treatment period died with relapsing disease during the next seven and one-half months.

Evidence of spread of disease to the cervical lymph nodes and the spleen was noted sixteen days after infection in untreated animals. Definite evidence of healing in the form of organization and fibrosis in the meningeal exudate and the cervical lymph nodes was seen twelve days after the onset of therapy.

SUMARIO

Meningitis Tuberculosa Experimental en el Cobayo: Resultados del Tratamiento con Iproniacida, Estreptomicina y Estreptomicina e Isoniacida

Prodújose meningitis tuberculosa experimental en cobayos por inoculación intracerebral de una pequeña cantidad de cepas humanas virulentas del *Mycobacterium tuberculosis*, H37Rv. Tratóse después de erradicar dicha infección por medio de la administración de varios distintos regímenes quimioterapéuticos, comprendiendo iproniacida sola, isoniacida sola, estreptomicina sola y una combinación de estreptomicina e isoniacida. Se estudió el efecto de la demora en el tratamiento, iniciando la droga un día u ocho o dieciséis días después de la infección. Un pequeño grupo de animales no fué tratado sino después de haber ya signos bien definidos de enfermedad. Todo tratamiento fué discontinuado a los seis meses de la infección, a continuación de lo cual se mantuvo a los animales sobrevivientes en observación durante otros siete meses y medio.

Los resultados de este experimento indican que, a las dosis usadas, la iproniacida, la isoniacida, la estreptomicina y la combinación de estreptomicina e isoniacida eran capaces de dominar muy bien la meningitis experimental en cuestión, con tal que se iniciara el tratamiento en término de ocho días de la infección. Con una tardanza de dieciséis días, la protección otorgada por dichas drogas no era absoluta y si se aplazaba el tratamiento hasta la iniciación de enfermedad bien definida, no resultaba beneficioso. Todos los cobayos no tratados murieron de dieciséis a veinticuatro días después de la infección.

Todos los regímenes medicamentosos usados dieron igual beneficio, en lo que pudo determinarse comparando las cutirreacciones a la tuberculina, el tiempo de sobrevivencia, la tasa de recidivas y la histopatología.

Ninguno de los regímenes terapéuticos mantenidos durante seis meses resultó suficiente para erradicar por completo los bacilos tuberculosos, dado que, excepto 2, todos los 36 animales que sobrevivieron el período completo de tratamiento, murieron de recurrencia de la enfermedad durante los próximos siete meses y medio.

En los animales no tratados, notáronse signos de difusión de la dolencia a los ganglios linfáticos cervicales y al bazo a los dieciséis días de la infección. A los doce días de la iniciación de la terapéutica, observáronse signos bien definidos de cicatrización, en forma de organización y de fibrosis, en el exudado meníngeo y los ganglios linfáticos cervicales.

RESUME

Méningite tuberculeuse expérimentale chez le cobaye; Résultats du traitement par l'isoniazide, l'iproniazide, la streptomycine ou la streptomycine et l'isoniazide

La méningite tuberculeuse expérimentale a été produite chez des cobayes par inoculation intracérébrale d'un petit nombre de *Mycobactérium tuberculosis H 37Rv.*, souche humaine virulente. Il a été tenté de juguler cette infection par l'administration régimes thérapeutiques différents comprenant l'iproniazide seul, l'isoniazide seul, la streptomycine seule et l'association de streptomycine et d'isoniazide. L'action du délai dans le traitement a été étudiée en administrant le composé soit un, huit ou seize jours après l'inoculation. Chez un petit groupe d'animaux le traitement n'a commencé que lorsque les signes définis de la maladie sont devenus évidents. Tout traitement était interrompu six mois après l'inoculation, après quoi les animaux survivants étaient observés pendant encore sept mois et demi.

Les résultats de cette expérimentation indiquent que, aux doses utilisées, l'iproniazide, l'isoniazide, la streptomycine et l'association streptomycine-isoniazide étaient capables de bien contrôler cette méningite expérimentale, à condition que le traitement soit instauré dans les huit jours suivant l'inoculation. Lorsque le délai était de seize jours la protection offerte par ces agents était incomplète, et si le traitement était retardé jusqu'au début de l'affection déclarée, il devenait inopérant. Tous les cobayes non traités ont succombé seize à vingtquatre jours après l'inoculation.

Tous les régimes thérapeutiques utilisés se sont montrés également favorables, dans la mesure où ils ont pu être évalués par la comparaison des tests cutanés à la tuberculine, des temps de survie, des taux de rechute et de l'histo-pathologie.

Aucune des méthodes de cure poursuivies pendant six mois n'a été suffisante pour obtenir l'éradication totale du bacille tuberculeux car, parmi les 36 animaux qui ont survécu pendant toute la période de traitement, tous sauf deux, sont morts à la suite de rechutes de l'affection au cours des sept mois et demi suivants.

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A FURTHER STUDY OF VIRULENCE IN GUINEA PIGS OF ISONIAZID-RESISTANT TUBERCLE BACILLI ISOLATED FROM CLINICAL MATERIAL[1, 2]

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The incidence of isoniazid-resistant tubercle bacilli isolated from tuberculous patients has been shown to be high (1, 2, 3). Investigations of the properties of isoniazid-resistant tubercle bacilli have therefore become of primary importance in the laboratory as well as in the clinic.

This report deals with the virulence for guinea pigs of resistant cultures of tubercle bacilli isolated from sputum of patients treated with isoniazid for six to nine months.

METHODS AND MATERIALS

Fifteen different strains of tubercle bacilli, resistant to at least 100 γ of isoniazid per ml. of Peizer-Schechter agar were isolated from 15 patients under treatment with isoniazid. These strains were first isolated on medium containing 5 γ of isoniazid per ml. and then subcultured once on the solid medium agar containing 100 γ of the drug per ml., and used for injection into guinea pigs. Fourteen-day-old culture growths were emulsified in phosphate buffer pH 7.0. After settling, the 15 smooth supernatant supensions of cells were adjusted to the same density, corresponding to 3×10^8 organisms per ml. Then, 0.25 ml. of each suspension was injected subcutaneously into the groin of each of 2 guinea pigs. Fifteen cultures susceptible to 0.1 γ of isoniazid per ml. of medium, isolated from the same patients before treatment with isoniazid, were also injected at the same time into guinea pigs, using one animal for each drug-susceptible culture. The drug-susceptible cultures were prepared for injection by the same procedure which was used for the resistant cultures, and animals were infected with the same number of bacterial units.

The guinea pigs weighed 350 to 400 gm. and were tuberculin-negative when tested intracutaneously with 0.1 ml. of 1:100 dilution of Old Tuberculin.

RESULTS

The tuberculin reactions of the infected guinea pigs are summarized in table 1. It may be seen that the 15 guinea pigs infected with drug-susceptible organisms all became tuberculin-positive within five weeks. The tuberculin reaction of the 30 guinea pigs injected with drug-resistant organisms became positive in 27 animals, but much later than in those infected with drug-susceptible cultures. Three of the 30 remained tuberculin-negative throughout the experiment, although the companion animal in each case became tuberculin-positive (*see* groups *D*, *E*, and *L*, figure 1). This indicates a difference in body response of the infected animals rather than an inability of the resistant organisms to initiate a reaction.

Of 12 animals which are still alive at present, 2 belong to the group of 3 which remained tuberculin-negative. Of the remaining 10 animals, 8 reverted from tu-

[1] From the Bureau of Laboratories, Department of Health, New York City.

[2] Presented at the meeting of the American Public Health Association on November 13, 1953.

TABLE 1

TUBERCULIN REACTION OF GUINEA PIGS INFECTED WITH EQUAL AMOUNTS OF DRUG-SUSCEPTIBLE AND DRUG-RESISTANT TUBERCLE BACILLI

Number of Guinea Pigs Infected	Number of Tuberculin-Positive Guinea Pigs	Number of Guinea Pigs Dead Within 12 Weeks	Number of Guinea Pigs Tuberculin-Positive at 24 Weeks	Number of Guinea Pigs Sacrificed at 24 Weeks	Number of Tuberculin-Positive Guinea Pigs Alive at 26 Weeks	Number of Guinea Pigs Reverted from Tuberculin-Positive to Tuberculin-Negative at 30 Weeks
Susceptible						
15	15 (4–5 weeks)	15	—	—	—	—
Resistant						
30	27 (4–16 weeks) 3*	3	24 3*	14 T.P. 1* 2*	10 2*	8 (2 T.P.) 2*

* —remained tuberculin-negative.
T.P.—tuberculin-positive.

berculin-positive at twenty-six weeks to tuberculin-negative at thirty weeks after infection. It is of interest to note that, although 14 of the 15 guinea pigs which were sacrificed at the end of twenty-four weeks were at that time tuberculin-positive, only 4 of the 14 had any gross lesions of tuberculous infection, consisting in each case of a small localized abscess at the point of inoculation (*see* groups *D*, *F*, *K*, and *M*, figure 1).

Results of the virulence tests six months after infection of the guinea pigs may be seen in figure 1. It will be noted that all animals infected with drug-susceptible organisms died within twelve weeks and, at necropsy, presented typical gross tuberculous lesions which were positive for tubercle bacilli on microscopy and culture.

The guinea pigs infected with resistant strains did not develop tuberculosis, with the exception of 3 which died of mixed infections. Eleven of 15 sacrificed animals infected with resistant strains presented no evidence of infection. The remaining 4 had only small local abscesses at the sites of injection. Approximately twelve weeks after infection, 2 of these 4 guinea pigs had developed large draining abscesses in the groins, from which tubercle bacilli were isolated on culture. Drug-resistance tests on these cultures indicated that the organisms retained a resistance to 100 γ of isoniazid per ml. of medium. These abscesses in the groin proceeded to shrink and heal until, at necropsy, twenty-four weeks after infection, they consisted of localized small flat pockets of caseous material with about one acid-fast bacillus in every 10 fields and 1 to 3 colonies on culture. There was no evidence of any lymphatic involvement in these 4 animals. All of the 15 animals which were sacrificed continued to gain weight and had tuberculin-positive reactions until they were sacrificed at twenty-four weeks.

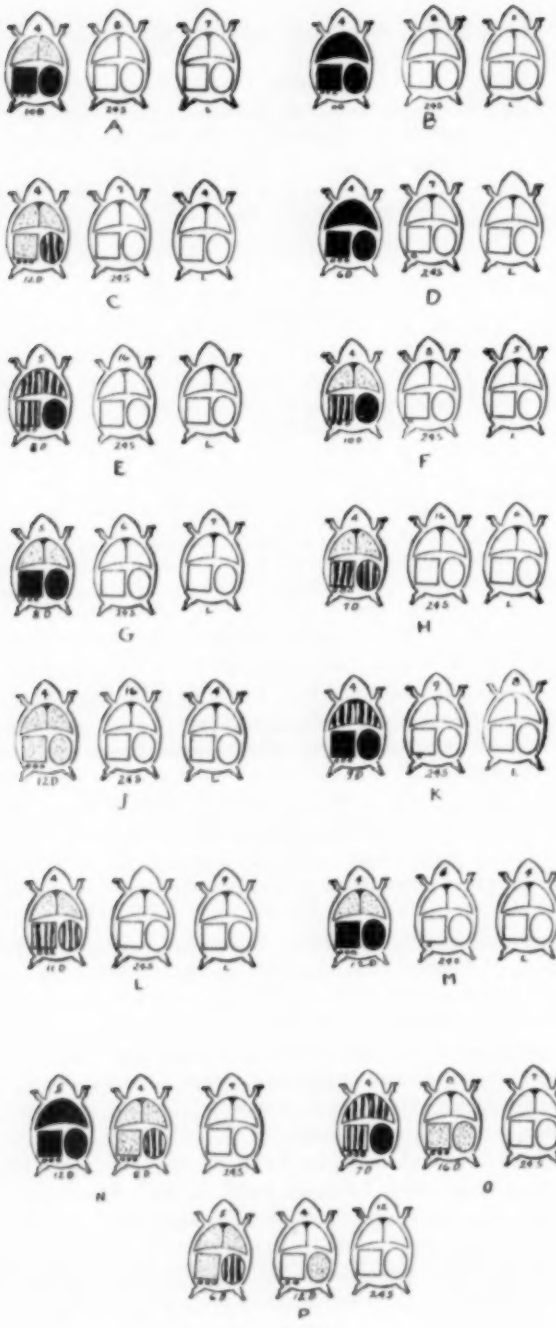

FIG. 1

The 3 animals which died, although infected with resistant organisms, presented at necropsy typical but not extensive tuberculous lesions (groups *N*, *O*, and *P*, figure 1). However, these animals also developed an enteric infection caused by a gram-negative bacillus which, when injected intraperitoneally into 3 normal guinea pigs, caused a continuous gradual loss of weight and death in fourteen weeks of one of the 3 animals. Subcultivation from the tuberculous lesions of the 3 animals which died of the mixed infection produced cultures of tubercle bacilli resistant to 100 γ of isoniazid per ml. of medium. The 3 companion animals which did not develop any concomitant disease showed no signs of tuberculosis when sacrificed twenty-four weeks after infection.

DISCUSSION

These results, showing almost a total loss of virulence of isoniazid-resistant cultures of *M. tuberculosis* for normal guinea pigs, seem to be at variance with results obtained by the present writers in an earlier study of virulence of isoniazid-resistant cultures for guinea pigs (4). In the earlier experiment, which was terminated at the end of fifteen weeks, 7 of 25 animals infected with resistant tubercle bacilli died of tuberculosis within twelve weeks; the remaining 18 were sacrificed. Of these, 10 had varying degrees of gross tuberculous infection of the internal organs and 8 had abscesses at the sites of inoculation, 4 with necrotic regional lymph nodes.

All animals infected with drug-susceptible tubercle bacilli died within three months in both studies. The varied results obtained with resistant organisms in the two studies may therefore be due to the different methods used in each experiment in culturing the organisms before injection into animals. In the earlier experiment, the primary cultures were isolated from concentrates on solid medium without any drug. Subcultures were then made once to Proskauer-Beck medium containing no drug. In the present study, the primary cultures were isolated from concentrates on agar containing 5 γ of isoniazid per ml. and transplanted once to solid medium containing 100 γ of isoniazid per ml. This was done to exclude any drug-susceptible organisms which might be present in the specimens. For it is known from *in vitro* studies done in this (5) and other laboratories (6) that populations of tubercle bacilli which are present in sputum consist of mixed organisms with varied degrees of resistance. Reversion of isoniazid-resistant organ-

FIG. 1. Extent of tuberculosis in guinea pigs at necropsy after injection with drug-susceptible and -resistant cultures of tubercle bacilli. The first animal diagram in each series of 3 animals infected with cultures isolated from the same patient represents the control (injected with susceptible culture). The other two represent animals injected with like amounts of resistant culture. The oval and rectangle represent the spleen and liver, respectively. The circles from right to left represent the site of inoculation and the regional lymph nodes. The extent of tuberculosis in the organs is indicated by the degree of shading; complete shading indicating maximal involvement, and stip ling minimal involvement. The numerals at the head indicate the number of weeks after injection when the animal became tuberculin-positive. The numeral and letter *D* indicate number of weeks of infection before death, and the numeral and letter *S* indicate the number of weeks after injection when the animal was sacrificed. The letter *L* indicates animals still alive.

isms to lesser resistance and to isoniazid-susceptibility has also been shown to occur (7). The exclusion of drug-susceptible organisms in the second study may at least partially explain the differing results in the two studies.

It is also seen here, incidentally, that isoniazid-resistant tubercle bacilli, although not virulent for normal guinea pigs, can produce tuberculous infection of the internal organs in animals which develop a concomitant disease causing internal necrotic lesions. Middlebrook and Cohn (7), in discussing the cultural requirements of resistant tubercle bacilli, refer to such a possibility in the following statement: "It is tempting to postulate that most strains of tubercle bacilli, which are resistant to more than 10 μg of INH/ml of artificial medium have a special growth requirement for a substance or substances, which on the one hand, is present but bound and unavailable in normal tissue, but on the other hand, free and available to these fastidious strains in necrotic tissue."

SUMMARY

Fifteen guinea pigs which were infected with fifteen different isoniazid-susceptible cultures of tubercle bacilli isolated from clinical specimens died of tuberculosis within twelve weeks. Thirty guinea pigs were infected with fifteen different strains of isoniazid-resistant tubercle bacilli isolated from the same tuberculous patients after six to nine months of treatment with isoniazid. Eleven of the 30 animals did not show any lesions when sacrificed twenty-four weeks after infection; 4 had localized small abscesses at the point of inoculation, but no glandular involvement. Three of the 30 animals developed a concomitant disease and died of mixed infections; these animals had some, but not extensive, tuberculous lesions of the internal organs. Twelve of the 30 animals are still alive at the present time.

All of the animals which were infected with isoniazid-susceptible organisms became tuberculin-positive within five weeks. Twenty-seven of the 30 animals injected with resistant organisms became tuberculin-positive in five to sixteen weeks. Three remained tuberculin-negative; 8 of the guinea pigs still alive, which were tuberculin-positive at twenty-six weeks, reverted to tuberculin-negative by the end of thirty weeks after infection with isoniazid-resistant organisms.

SUMARIO

Estudio Ulterior de la Virulencia en los Cobayos de los Bacilos Tuberculosos Isoniacidorresistentes Aislados de Ejemplares Clínicos

Quince cobayos que fueron infectados con quince distintos cultivos isoniacidosusceptibles de bacilos tuberculosos aislados de ejemplares clínicos murieron de tuberculosis en término de doce semanas. De 30 cobayos infectados con quince distintas cepas de bacilos tuberculosos isoniacidorresistentes aislados de ejemplares procedentes de los mismos enfermos al cabo de seis a nueve meses de tratamiento con isoniacida, 11 no presentaban lesión alguna al ser sacrificados veinticuatro semanas después de la infección; 4 tenían pequeños abscesos localizados en el punto de inoculación, pero sin invasión ganglionar; 3 de los 30 manifestaron una afección concomitante y murieron de infecciones mixtas con algunas, pero no extensas, lesiones tuberculosas de las vísceras. Doce de los 30 animales se hallan todavía vivos a esta fecha.

Todas los animales infectados con microbios isoniacidosusceptibles se volvieron tuber-

culino-positivos en término de cinco semanas. Otro tanto sucedió en término de cinco a dieciséis semanas en 27 de los 30 animales en que se inyectaron microbios resistentes. Tres permanecieron tuberculino-negativos; 8 de los cobayos todavía vivos, que eran tuberculino-positivos a las veintiséis semanas, volvieron a ser tuberculino-negativos al cabo de treinta semanas de la infección con microbios isoniacidorresistentes.

RESUME

Etude complémentaire de la virulence des bacilles tuberculeux isoniazide-résistants isolés de spécimens cliniques chez le cobaye

Quinze cobayes, ayant été inoculés à l'aide de quinze cultures différentes de bacilles tuberculeux isoniazide-sensibles isolés à partir de spécimens cliniques, sont morts de tuberculose en douze semaines. Sur un nombre de 30 cobayes inoculés avec 15 souches différentes de bacilles tuberculeux isoniazide-résistants isolés de spécimens prélevés chez ces mêmes malades après six à neuf mois de traitement par l'isoniazide, 11 ne présentaient aucune lésion quand ils ont été sacrifiés vingt-quatre semaines après l'inoculation; 4 avaient de petits abcès localisés au point d'inoculation, mais sans atteinte des ganglions lymphatiques; 3 parmi les 30 ont été affectés d'une maladie concomitante et ont succombé à des affections mixtes; il existait quelques lésions tuberculeuses peu étendues des organes internes. Parmi ces 30 animaux, 12 sont encore vivants actuellement.

La totalité des animaux qui avaient été inoculés avec des bacilles isoniazide-sensibles a présenté une réaction positive à la tuberculine dans les cinq semaines suivantes. Sur les 30 animaux inoculés avec les organismes résistants, 27 ont présenté une réaction positive à la tuberculine dans les cinq à seize semaines suivantes. Trois animaux sont restés anergiques à la tuberculine; 8 des cobayes encore vivants, et chez lesquels la réaction à la tuberculine était positive à la vingt-sixième semaine, sont redevenus anergiques à la tuberculine au bout de trente semaines après inoculation avec les bacilles isoniazide-résistants.

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Case Report

TUBERCULOUS CHOLECYSTITIS[1,2]

Report of a Case and Review of the Literature

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Tuberculous involvement of the gallbladder is a rare pathologic finding and is infrequently described in the literature. The first case was described by Gaucher (1, 2) in 1870, and an extensive review by Rankin and Massie (3) in 1926 revealed a total of fifteen cases to that time. Since then isolated cases have been reported, the most recent being a case of tuberculosis of the liver and gallbladder by Leader (2). As mentioned by him, only thirty-seven cases of tuberculous cholecystitis had been reported by 1952. Elfring (4), in 1949, stressed the revealing fact that less than one-half of the cases reported had been proved histologically.

The following is presented as another case of tuberculosis of the gallbladder. It was unusual in that there was no evidence of a tuberculous process involving any of the other intra-abdominal organs. The diagnosis in this instance was made entirely on the gross and microscopic pathology. Attempts to isolate tubercle bacilli from the patient were unsuccessful.

CASE REPORT

A 73-year-old American Indian woman was admitted to the hospital on April 14, 1953, with a ten-year history of fatty food intolerance, accompanied by right upper quadrant abdominal pain which radiated to the angle of the right scapula. These attacks were associated with nausea and vomiting. For one and one-half years prior to admission, these episodes had become more severe, lasting one week and recurring approximately once a month. No history of jaundice, clay-colored stools, or dark urine was elicited. The patient denied ever having had tuberculosis or having been treated for any lung disease. The physical examination was essentially normal. The clinical impression was that of chronic cholecystitis with cholelithiasis.

A chest roentgenogram (figure 1) revealed a small area of calcification in the right apex which was interpreted as healed tuberculosis. A cholecystogram was performed and no filling of the gallbladder was demonstrated (figure 2). In the right upper quadrant, however, a diffuse area of calcification was noted which appeared to outline the gallbladder. The roentgenographic diagnosis was nonfunctioning gallbladder with calcification within its wall. Chemical determinations of various components of the blood, including liver function tests, all yielded normal values.

A surgical exploration of the abdomen was made by one of the writers (R. A. G.). The wall of the gallbladder was found to be thickened and calcified, and stones could be palpated within it. The common duct was grossly normal, and no stones were palpated within its entire length. No abnormal lesions of the liver or gallbladder bed were noted. Exploration of the entire abdominal cavity revealed no abnormality other than that of the gallbladder.

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[2] The opinions expressed are those of the writers and do not necessarily reflect those of the United States Public Health Service or the Office of Indian Affairs.

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FIG. 1. Chest roentgenogram showing old healed tuberculous lesion in the right apex

FIG. 2. Roentgenogram showing the calcification in the wall of the gallbladder

A routine cholecystectomy was performed. After the operation, the gallbladder was opened and was found to contain a thick, caseous, cheesy yellowish-white material which completely filled the lumen. Several stones were present within this material. The pathologic report of the gross specimen showed the gallbladder wall to be 2 to 3 mm. thick with calcification of its lining. The lumen was filled with thick, inspissated, pasty yellowish-white material and contained a 2 cm. concretion with several smaller fragments. Microscopically, the lining of the lumen consisted of amorphous esophinophilic material overlying reticulo-

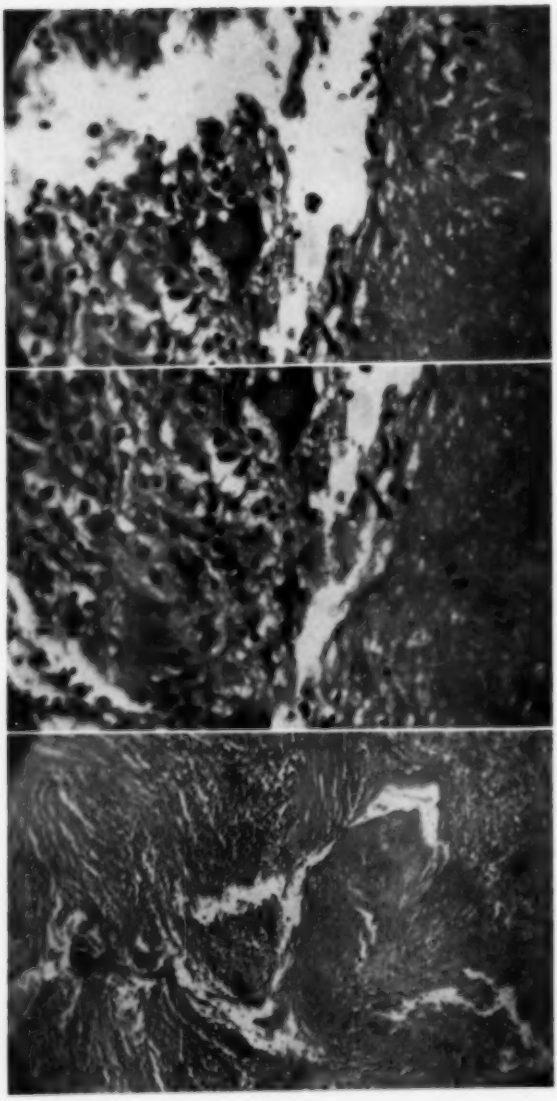

FIG. 3. *A*. Low-power field of the wall of the gallbladder showing caseation and Langhans' giant cells. *B*. High-power field of the wall of the gallbladder showing caseation and Langhans' giant cells. *C*. High-power field of the wall of the gallbladder showing caseation and Langhans' giant cells.

endothelial cells. These were intermixed with multinucleated giant cells and infiltrated with lymphocytes. Foci of calcification were noted in the deeper layers surrounded by reticuloendothelial and multinucleated giant cells (figure 3). The diagnoses were: (*1*) fibrocalcific granulomatous cholecystitis consistent with tuberculosis, (*2*) cholelithiasis.

After reviewing the pathologic report, three gastric aspirations and duodenal intubation were done to obtain material for culture. The smears and cultures of these specimens were negative for tubercle bacilli. The patient left the hospital before a course of antituberculous therapy could be given.

COMMENT

A number of theories have been proposed in an attempt to account for the infrequent localization of tuberculosis in the gallbladder (5). Foremost of these appear to be the increased resistance of the gallbladder mucosa and the adverse effect of bile on the tubercle bacilli. Sergent (6) demonstrated that tubercle bacilli introduced into the gallbladder of guinea pigs and dogs did not produce lesions unless the mucosa was injured or the common duct ligated. He suggested that the fat-splitting and dissolving properties of bile and pancreatic juices might possibly alter the fatty coat of the tubercle bacillus.

Different routes have been described by which the gallbladder may become infected with the tubercle bacillus (1, 3, 5, 7). These are:

1. Hematogenous dissemination from the primary focus with involvement from the gallbladder
2. Spread from the liver through the biliary tree
3. Direct extension from the liver
4. Secondary involvement from a tuberculous peritonitis
5. The remote possibility of extension upward from the duodenum via the common bile duct

As, in the present case, the gallbladder was the only intra-abdominal organ involved with the tuberculous process, it can be postulated that the original infection took place when the hematogenous dissemination from the primary focus occurred and that it did not come about by direct contiguity from any of the other abdominal organs.

The diagnosis of tuberculosis of the gallbladder prior to operation or autopsy is extremely difficult to make. Only two cases have been described in the literature (3, 8) in which the diagnosis was made preoperatively. Most of the cases described occurred in women more than thirty years of age with the presenting symptoms of patients with acute or chronic cholecystitis (1, 2). Approximately one-half of the reported cases were associated with cholelithiasis (1, 2, 3). In general, the age and sex incidence and signs and symptoms parallel those of patients with chronic gallbladder disease.

SUMMARY

A case of a patient with typical signs and symptoms of chronic cholecystitis is presented. The gross and microscopic pathology were consistent with tuberculosis of the gallbladder and cholelithiasis.

SUMARIO

Colecistitis Tuberculosa

Preséntase el caso de un enfermo que tenía típicos signos y síntomas de colecistitis crónica. La histopatología macro y microscópica era compatible con tuberculosis de la vesícula biliar y colelitiasis.

RESUME

Cholécystite tuberculeuse

Le cas d'un malade présentant les manifestations pathologiques et symptômes caractéristiques de la cholécystite chronique est présenté. Les lésions macroscopiques et histopathologiques confirmaient le diagnostic de tuberculose de la vésicule biliaire avec cholélithiase.

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Editorial

A Point of View About Bacterial Resistance[1]

U. S. Public Health Service Cooperative Clinical Investigation

Bacterial resistance is generally regarded as the limiting factor in the use of the available antimicrobial agents against tuberculosis. It might also be looked upon as a psychological block: the mere fact that drug-resistant bacilli can appear exerts a subtle influence on our day-to-day decisions about the use of the various agents. The consequences for the patient are perhaps more feared than known. In this situation, it seems appropriate to try to see what attitude the evidence justifies.

The introduction of chemotherapy into the treatment of tuberculosis has presented to the laboratory a multitude of new and fascinating problems for investigation, and research has increased many fold during the last decade. As a result, a great deal of interesting and valuable information on bacterial resistance in the test tube and in experimental animals has become available. But, the meaning of these findings for the clinical care of *tuberculous human beings* is far from clear.

This situation is not difficult to understand. Laboratory techniques and language have become increasingly complex and specialized. The clinician, finding himself less than expert in interpreting *in vitro* observations, is nevertheless in awe of pronouncements emanating from the laboratory. The bacteriologist has come to be regarded as something of an oracle. Misinterpretations and misapplications of perfectly sound laboratory findings are thus almost inevitable. Consequently, although there is now a considerable body of knowledge from the laboratory about bacterial resistance, we still understand very incompletely how, as clinicians, we should best use this information in treating our patients.

Reasoning from observations of the tubercle bacillus' behavior in the Petri dish to conclusions about its action in the human host entails a certain risk. The most nutritious medium, the optimal temperature, and the tender loving care of the laboratory staff may so alter the organism's natural environment that it occasionally shows an unusual capacity for survival. We have no notion how frequently, or rather how rarely, it shows such ability during its struggle for existence and reproduction in the human being, surrounded by phagocytes and powerful chemotherapies.

To obtain knowledge of the effect of bacterial resistance in man, it seems sensible to observe human beings directly and to see what actually happens to tuberculous patients treated with the available drugs. Our studies have led us to hold certain views on who is at risk, that is, in which patients bacterial re-

[1] The material in this editorial was presented before the Medical Session, as part of the session on *Medical Treatment of Tuberculosis*, at the annual meeting of the National Tuberculosis Association, Atlantic City, New Jersey, on May 20, 1954.

sistance appears, and on the nature of the relation between the presence of drug-resistant bacilli and the clinical course of the disease.

In the Public Health Service studies of antimicrobial therapy, large numbers of patients have been treated with various combinations of drugs. And, as in all such investigations, there has been some anxiety about the bacterial resistance which might be encountered on new regimens and with new drugs. An attempt has been made during these studies to make observations which might provide information on resistance. The attempts have not been entirely successful and the results were more meager than had been hoped. One thing, at least, was established with certainty: An enormous number of patients are required for a really definitive study of bacterial resistance. It is not likely, therefore, that many investigators or even groups of investigators will have sufficient material, and we, in the breach, offer some findings from our "too-small" data.

A full description of how this material has been collected has been presented elsewhere (Am. Rev. Tuberc., 1953, *68*, 264). In these studies, in which more than twenty hospitals throughout the country have participated, great emphasis has been placed on objectivity and uniformity. In planning the studies, treating the patients, making and recording observations, and analyzing the results, every effort has been made to observe the principles which would give unbiased information.

The present report is based on material from an investigation of three regimens: isoniazid alone, isoniazid–streptomycin, and streptomycin–PAS. Five hundred and eight-three patients who were discharging tubercle bacilli susceptible to both streptomycin and isoniazid at the beginning of the study remained under observation for forty weeks. All of the drug-susceptibility tests were performed in the Trudeau Laboratory on cultures sent by the individual hospitals.

A detailed analysis of the material on these 583 patients has been completed. Without presenting the details, it is possible to form a quite convincing story by piecing together all evidence which has been gathered. That story points fairly clearly to the conclusion that bacterial resistance has been causing greater concern than it merits.

After forty weeks of treatment, very few cultures still contained only organisms susceptible to the drugs used: on isoniazid treatment alone, there were none; on isoniazid-streptomycin treatment, 1 per cent was susceptible to isoniazid and 4 per cent were susceptible to streptomycin; on streptomycin–PAS therapy, 4 per cent were susceptible to streptomycin. Although the three regimens were not equally effective in producing bacteriologic conversion, nearly all of the cultures still positive for *M. tuberculosis*, whatever the therapy, had become resistant. Thus, if tubercle bacilli are continually discharged during prolonged chemotherapy, the organisms nearly always develop resistance to the particular agents to which they have been exposed.

Everyone has been interested in whether certain conditions favor the emergence of resistant organisms. Now, it is evident that the question "which patients will have drug-resistant bacilli?" is really the same question as "which patients will continue to produce 'positive' sputum?"

To answer the question, the patients have been classified according to certain characteristics of their disease, and the proportion of each disease group with tubercle bacilli in the sputum after forty weeks of treatment has been computed. This was done separately for each regimen. And, on each regimen, the *more extensive the cavitation*, the *older the disease*, and the *more advanced the stage*, then the more frequently tubercle bacilli continued to be present in the sputum and, consequently, to be resistant to the drugs used.

These same factors, *cavitation*, *duration*, and *stage of disease*, also affect the frequency with which patients show roentgenographic improvement. Patients with far advanced cavitary disease of long duration are those who most frequently fail to improve roentgenographically. Not only do these categorizations, *failure to become "sputum negative"* and *failure to show improvement on the chest roentgenogram*, both occur most frequently in patients of this type, but the patients who fail to show roentgenographic improvement are, in fact, those who continue to discharge tubercle bacilli and, therefore, harbor resistant organisms.

A number of workers have noted an association between the detection of drug-resistant bacilli and clinical progression or relapse, and from it, some have concluded that the resistance of the organisms was necessarily the cause of the failure to improve, and that, therefore, the development of resistance was always harmful to the patient.

However, their assumption of a universally operative cause-and-effect relationship does not take into account the critical point, that these two conditions are associated chiefly in a *particular kind of case*. When this fact is considered, it is quite as logical to take the view that failure to become bacteriologically negative (and, hence, to harbor resistant tubercle bacilli) and failure to demonstrate roentgenographic improvement are to be expected in some cases of long-standing, extensive cavitary disease—cases for which our present-day chemotherapy is least effective.

One other point should be considered: how *frequently* do drug-resistant strains of tubercle bacilli appear?—in practically all of the cultures that remain positive. *But*, how frequently do cultures remain positive for tubercle bacilli? There is a tendency to forget that, when we speak of the patients "whose cultures remain positive," we are speaking today of a relatively small group. Before chemotherapy, *most* patients discharged tubercle bacilli in the sputum for long periods of time. The situation is very different today—most patients *do not* continue to discharge bacilli in the sputum. For instance, with the most effective regimen in the PHS study, isoniazid–streptomycin, the cultures of 80 per cent of the patients were negative for *M. tuberculosis* at the end of the fortieth week of treatment.

The primary aim of therapy, bacteriologically, is certainly the *elimination* of the bacilli. Preserving the drug susceptibility of organisms that persist despite prolonged treatment is only a *secondary* consideration, desirable if the primary objective of getting rid of the organisms is not accomplished. It is now known that, with present-day chemotherapy, tubercle bacilli will disappear from the sputum of all but a small proportion of the patients treated. This small group is

composed chiefly of patients with far advanced cavitary disease of long duration, and for them there has not yet been found a way to use the drugs without the development of drug-resistant organisms. Bacterial resistance appears in these cases and, it is believed, represents merely one of several manifestations of a generally unfavorable response to drug treatment for tuberculosis. In searching for more effective treatment, the search should be directed toward a therapy or combination of therapies which will benefit this small group—the present treatment failures.

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Notes

PYRAZINAMIDE–ISONIAZID IN TUBERCULOSIS[1, 2]

II. Results in 58 Patients with Pulmonary Lesions One Year After the Start of Therapy

In a previous report by the present writers[3], data were presented from a series of integrated studies of the antituberculous activity of pyrazinamide-isoniazid in man and experimentally infected mice. On the basis of three types of evidence, microbial enumeration in mice and roentgenographic and microbiologic studies in man, it was concluded that pyrazinamide-isoniazid was superior to the other antituberculous chemotherapies. It was further concluded that in mice the two drugs together exerted qualitatively unique effects of an "eradicative" type. The opinion was expressed, however, that the use of pyrazinamide-isoniazid on a widespread basis was inadvisable unless subsequent manipulation of dosage could appreciably lessen the risk of hepatitis.

In the previous report, the observations in man consisted of the roentgenographic and microbiologic results obtained *six months* after the start of treatment in a group of 55 patients with pulmonary tuberculosis and *nine-month* observations in approximately half of the group. As the entire group of 58 patients had passed the *twelve-month* observation point by mid-June, 1954, the results at that point have been analyzed and form the principal basis of the present report.

The group thus consists of 58 patients with pulmonary tuberculosis, all of whom received pyrazinamide-isoniazid for at least three months with isoniazid alone thereafter and all of whom were started on this treatment in the period from July 13, 1952, to June 19, 1953. The daily dosage of pyrazinamide was 50 mg. per kg.; the daily dosage of isoniazid was 5 mg. per kg. Thirteen of the patients received the pyrazinamide for approximately six months. The details concerning the patients and the methods used in the investigation are presented in the earlier report.[3]

Success in completing one year of therapy: Of the 58 patients who completed at least three months of the multiple-drug phase of the regimen, 41 completed the year without either a change in chemotherapy or the use of pneumoperitoneum or surgical therapy. An additional 4 patients, who had experienced substantial roentgenographic improvement and reversal of infectiousness, received additional types of chemotherapy on transfer to other hospitals in conformance with individual local practices.

The eliminations from the study occurred principally in the third quarter of the year and were made for the following reasons:

In the second quarter, 5 patients were eliminated by self-discharge.

In the third quarter, 11 patients were eliminated: 4 by transfer to other hospitals, 5 by pneumoperitoneum or surgery, and 2 by self-discharge.

[1] This study was supported in part by grants from the Navajo Tribal Council; the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service; grants from the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York; and Chas. Pfizer & Co., Inc., Brooklyn, New York.

[2] The pyrazinamide (Aldinamide, Lederle) and the isoniazid (Nydrazid, Squibb) used in this study were generously supplied by the manufacturers.

[3] McDermott, W., Ormond, L., Muschenheim, C., Deuschle, K., McCune, R. M., Jr., and Tompsett, R.: Am. Rev. Tuberc., 1954, *69*, 319.

In the fourth quarter, one patient was eliminated, and the reason was the performance of surgery.

Exclusive of the 6 patients treated with pneumoperitoneum or surgery, therefore, only 11 of the 58 patients who completed the first 90-day pyrazinamide phase of the regimen failed to complete the full year of chemotherapy.

Status at the time of collapse or excisional therapy: Of the 6 patients who received excisional or collapse therapy (including pneumoperitoneum) during the year, all had experienced substantial roentgenographic improvement and only 3 of the 6 were still discharging tubercle bacilli when these additional forms of treatment were instituted.

Roentgenographic course: Six months after the start of pyrazinamide-isoniazid therapy, the roentgenographic course of the 55 patients[4] for whom data were available was designated *substantial improvement* in 42 patients; *no significant change* in 13 patients; and *worse* in no patients.

Nine months after the start of therapy, the roentgenographic categorizations in the 42 patients for whom data were available were: *substantial improvement* in 34 patients; *no significant change* in 8; and *worse* in no patients.

Twelve months after the start of therapy, the roentgenographic categorizations in the 39 patients[5] for whom data were available were: *substantial improvement* in 31 patients; *no significant change* in 8 patients; and *worse* in no patients.

In 3 of the 8 patients with essentially unchanged roentgenographic findings, no *substantial* regression was possible because the lesions were minimal and became satisfactorily arrested; the other 5 patients had chronic advanced disease which appeared to be chiefly fibrotic in character.

Cavity closure (all cavities in each patient) as determined roentgenographically occurred in 28 of the 36 patients with clearly demonstrable cavities at the start of therapy. All but 5 of the 28 cavity closures occurred during the first half of the year of therapy.

Status of infectiousness: At three months after the start of pyrazinamide-isoniazid, *M. tuberculosis* was cultured from the sputum or gastric washings of 4 patients.

At six months, M. tuberculosis was cultured from only the same 4 patients (2 of the 4 then received collapse or excisional therapy).

At nine months, M. tuberculosis was cultured from 2 of the 42 patients available for microbiologic studies. These 2 were members of the group of 4 patients who had been discharging tubercle bacilli at all previous observation points, and one of the 2 was then treated with excisional therapy.

At twelve months, M. tuberculosis was cultured from only one of the 41 patients available for microbiologic studies. This one patient had been a member of the group of 4 just mentioned, and he represented the only one of the 4 who did not receive collapse or excisional therapy.

As noted in the previous report, there was good agreement between the results of microscopy and culture of the concentrated specimens of sputum or gastric washings.

Thus, except for 4 patients, the presence of tubercle bacilli in the sputum or gastric washings could not be demonstrated in the entire group at any time after a point relatively early (second or third month) in the pyrazinamide-isoniazid therapy. The 4 patients who represented exceptions behaved in diametrically opposite fashion. They all continued to discharge tubercle bacilli throughout the six to twelve months of chemo-

[4] Includes 2 patients eliminated at the end of the second quarter.

[5] Not included are 2 patients who completed the year of therapy under observation, but whose twelve-month roentgenograms were unavailable at the time of review.

therapy during which they were observed before the institution of collapse or excisional therapy. The pulmonary lesions of these 4 patients, as judged by roentgenography, were not distinctive in any way. Two of the 4 patients were diabetics, but the other 2 had no associated abnormal conditions.

* * *

The roentgenographic and microbiologic results one year after the start of the pyrazinamide-isoniazid therapy of the 58 patients were the same to all intents and purposes as when the same patients were evaluated after the first six months of the chemotherapy. Hence, it seems reasonable that the conclusion reached from the previous information should remain unchanged, namely, that pyrazinamide-isoniazid exerts antituberculous activity *in vivo* of a sort superior to that of the other current antituberculous drugs used in either single- or multiple-drug regimens.

It should be emphasized, however, as considered in detail in the previous report, that this conclusion is based on three types of evidence, all of which are considered to be equally essential for the conclusion. The third type of essential evidence, in addition to the roentgenographic and microbiologic studies in man, was provided by the microbial enumeration studies in the mice[3, 6, 7]. The reason for this belief that all three types of evidence are necessary was discussed in the previous report. It is based largely on the fact that it is difficult to distinguish among any multiple-drug regimens *which contain isoniazid* solely by roentgenographic and microbiologic studies of previously untreated patients[5, 9].

Pyrazinamide-isoniazid therapy cannot attain widespread usefulness unless it is possible to avoid or minimize the incidence of the toxic reaction of hepatitis. It seems reasonable to assume that the hepatitis is principally or entirely related to the pyrazinamide. Based on the published reports of 355 patients with pulmonary tuberculosis who have received pyrazinamide either alone or with one or another antituberculous drug,[4, 10–13] the incidence of icteric hepatitis has been 3.4 per cent.

The effects of alterations in the time-dose relationships of pyrazinamide have been under investigation by the present writers in both animals and man. Thus far, only fragmentary information is available as follows:

> First, as related in the previous report[3], the "eradicative" type action of pyrazinamide-isoniazid on tubercle bacilli in mice can be produced uniformly when the pyrazinamide is administered with the isoniazid for a period of 8 weeks (with isoniazid alone thereafter). When the pyrazinamide is administered for only 4 weeks, however, the "eradication" phenomenon cannot be produced with uniformity. Although there is no correlation between the results in the mouse and in man in terms of the total daily dosage of pyrazinamide, the time relationships of the "escape" from pyrazinamide influence are the same in the two species. Whether this means that the time rela-

[6] McCune, R. M., Jr., and Tompsett, R.: (In manuscript).

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tionships will prove to be the same in the mouse and in man with respect to the length of the period of pyrazinamide administration necessary for satisfactory effectiveness is obviously an open question.

Second, there were 6 patients who met the same criteria as the 58 patients of the present and previous reports but who received only 2.5 to 4.5 weeks of pyrazinamide-isoniazid with isoniazid alone thereafter. Five of these 6 patients were still discharging tubercle bacilli 90 days after the start of the chemotherapy. Only 4 of 58 patients were discharging tubercle bacilli at the same observation point in the series treated with at least 90 days of pyrazinamide with the isoniazid. Reversal of infectiousness eventually occurred in 3 of these 6 patients after 12, 21, and 28 weeks, respectively. The other 3 were still discharging bacilli 21 to 39 weeks after the start of chemotherapy. In contrast to the results in these 6 patients, there remained 4 patients who had received 10 weeks of pyrazinamide and another who had received 7.5 weeks when the use of the 50 mg. per kg. daily dose was abruptly discontinued. Reversal of infectiousness had occurred in all 5 of these patients between the fourth and seventeenth weeks after the start of chemotherapy.

Third, the use of a total daily dose of pyrazinamide of 25 to 30 mg. per kg. for a 10- to 13-week period with isoniazid concurrently and thereafter was started in January, 1954. To date approximately 25 patients with pulmonary tuberculosis have been treated on this regimen. Although the initial observations are comparable with the corresponding data for the higher pyrazinamide dosage, the information is insufficient to conclude that the lower dosage is a satisfactory one.

Fourth, all but one of the 12 reported cases of icteric hepatitis on pyrazinamide received a daily dose of 40 or more mg. per kg. of body weight. Lesser doses have actually been used, however, in only a relatively small number of patients. Consequently, there is virtually no information as to whether a pyrazinamide dosage of 30 to 35 mg. per kg. will be associated with an acceptably low incidence of hepato-toxicity.

The above information is obviously fragmentary and permits no definite conclusions. Nevertheless, it can be used as a basis for trial. The information suggests that a satisfactory pyrazinamide-isoniazid regimen might consist of: 10 to 13 weeks of the two drugs together from the therapeutic standpoint; and a total daily dosage of pyrazinamide of approximately 25 to 35 mg. per kg. from the standpoint of hepatic toxicity. The series of patients mentioned above who are being treated on this regimen is being steadily enlarged and will form the basis of a subsequent report. The series consists entirely of patients whose pulmonary tuberculosis is classified as far advanced by the National Tuberculosis Association 1952 classification.

Another important question concerning pyrazinamide-isoniazid, in addition to hepatic toxicity, is whether the effectiveness of the therapy is compromised by previous unsuccessful chemotherapy which included isoniazid. The present writers have had experience with only one such patient, and she has apparently derived no benefit from pyrazinamide-isoniazid. Schwartz and Moyer[13] emphasize that, in their experience, previous treatment with either isoniazid or pyrazinamide does substantially reduce the effectiveness of pyrazinamide-isoniazid.

In summary: The results one year after the start of chemotherapy are presented for a previously reported group of 58 patients with pulmonary tuberculosis who received pyrazinamide-isoniazid for at least three months with isoniazid alone thereafter. The roentgenographic and microbiologic results at the one-year observation point were essentially the same as reported for the earlier observation points. It is believed that the present roentgenographic and microbiologic results considered along with microbial enumeration studies in mice permit the conclusion that pyrazinamide-isoniazid exerts

antituberculous activity *in vivo* of a sort superior to that of the other current antituberculous drugs used in either single- or multiple-drug regimens.

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ACTIVATION OF PYRAZINAMIDE AND NICOTINAMIDE IN ACIDIC ENVIRONMENTS IN VITRO[1]

Pyrazinamide has no consistently demonstrable influence on *M. tuberculosis* growing under conventional conditions *in vitro*[2, 3]. Less detailed studies have been made with the parent compound, nicotinamide, but it apparently behaves in an identical fashion. Nevertheless, both pyrazinamide[4-6] and nicotinamide[7-10] have considerable antituberculous activity in laboratory animals, and pyrazinamide has impressive antituberculous activity in man[3, 11, 12]. Indeed, judged by the speed and uniformity of defervescence in tuberculous patients, pyrazinamide exerts its influence more quickly than isoniazid or the other available chemotherapies. Moreover, in quantitative studies by the microbial enumeration technique in mice, the magnitude of the antituberculous activity of pyrazinamide during the first four to six weeks of its administration is second only to that of isoniazid and in certain circumstances exceeds it[7, 8, 12].

In both man and the mouse, however, in many instances the tuberculous infection "escapes" from the influence of pyrazinamide after a variable period which ranges from three to six weeks[7, 8, 11].

This "escape" phenomenon has been considered to represent the emergence of a drug-resistant infection. As it is not possible by conventional tests to demonstrate any *drug susceptibility* of tubercle bacilli isolated either before or after pyrazinamide therapy, the presence of drug resistance cannot be confirmed in the usual way.

Several possibilities suggest themselves as explanations for this intriguing discrepancy between the *in vitro* and the *in vivo* antituberculous activities of pyrazinamide or its parent, nicotinamide.

One possibility is that these compounds are converted by the body into a substance with antimicrobial activity of the customary type. Attempts were made to study this possibility in human subjects, but were unsuccessful[12]. Although the experiments were

[1] This study was supported in part by grants from the Navajo Tribal Council; the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service; grants from Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York; and Chas. Pfizer & Co., Inc., Brooklyn, New York.

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[12] McDermott, W., Ormond, L., Muschenheim, C., Deuschle, K., McCune, R. M., Jr., and Tompsett, R.: Am. Rev. Tuberc., 1954, *69*, 319.

not so detailed as to exclude completely all possibilities of this sort, they provided no support for the notion that pyrazinamide exerted its effects by being transformed into a substance with readily demonstrable *in vitro* activity. Moreover, in experiments in mice, it was found that the tissues of pyrazinamide-treated animals had no inhibitory effect on tubercle bacilli when tested *in vitro*.

Accordingly, attempts were made to investigate another possibility, namely, that pyrazinamide and nicotinamide were capable of exerting antimicrobial activity in the biochemical environment of tuberculous infection but not in the "physiologic" conditions of *in vitro* testing.

The particular component of the environment chosen for initial study was its degree of acidity, and the results obtained form the basis of the present report.

All of the experiments were conducted according to the same general plan. Observations were made of the effects of a graded series of concentrations of pyrazinamide or nicotinamide on *M. tuberculosis* at eight different hydrogen ion concentrations from pH 4.5 to pH 8.0. Measurements of the respective activities of isoniazid and streptomycin were likewise made in the same experimental system.

In most instances the experiments were performed in duplicate in Tween®-albumin and oleic-acid–albumin liquid media. The strains of *M. tuberculosis* used were the H37Rv and the Ravenel strains of human and bovine origin, respectively. Unless otherwise stated, inocula were prepared from seven-day growth in Tween®-albumin medium and consisted of 0.02 ml. of culture or approximately 1×10^6 viable units inoculated into each 5 ml. volume of medium. This inoculum is the one used routinely in drug-susceptibility tests in this laboratory. Adjustment of the two media to the desired pH was made by sodium hydroxide or hydrochloric acid. The cultures in the various experimental series were maintained at 37°C. and were inspected weekly, usually for a total period of two weeks. The values were recorded in terms of growth visible macroscopically after the two-week period of incubation. The pH of uninoculated tubes of media adjusted to the various hydrogen ion concentrations used in the experiments was determined during comparable incubation and remained without significant change. The results of a series of representative experiments may be seen in tables 1 to 5.

In the first experiment (table 1) the influence of pH on the antimicrobial activity of pyrazinamide on tubercle bacilli of human origin (H37Rv) was determined. In the neigh-

TABLE 1

ACTIVITY OF PYRAZINAMIDE AGAINST H37RV TUBERCLE BACILLI ACCORDING TO ENVIRONMENTAL ACIDITY

(*Inoculum* of standard size; *media*, oleic-albumin and Tween®-albumin; *activity* measured by macroscopically visible bacillary growth at two weeks)

pH	Drug, γ per Ml.							
	503	250	125	63	32	16	8	0
8.0		0	0	±	4+	4+	4+	4+
7.5		±	2+	4+	4+	4+	4+	4+
7.0		2+	4+	4+	4+	4+	4+	4+
6.5		1+	3+	4+	4+	4+	4+	4+
6.0		0	0	±	4+	4+	4+	4+
5.5		0	0	0	0	0	1+	4+
5.0		0	0	0	0	0	±	3+
4.5								0

borhood of neutrality (pH 6.5, 7.0, and 7.5), the tubercle bacilli grew in the presence of the highest concentrations of pyrazinamide tested (250 γ per ml.). In an alkaline environment (pH 8.0), some pyrazinamide activity was detectable, for the bacilli failed to grow in drug concentrations of 125 and 250 γ per ml. In the more acidic environments, however, the antimicrobial action of pyrazinamide became impressive and increased markedly in degree with increases in the environmental acidity. Thus, the concentrations of pyrazinamide per milliliter of medium which completely inhibited growth of the tubercle bacilli were 125 γ at pH 6.0; 16 γ at pH 5.5; and 15 γ at pH 5.0; and, at the last-named pH, 8 γ per ml. produced almost complete inhibition of visible growth.

Repetitions of these observations with pyrazinamide and H37Rv yielded essentially the same results (tables 2 and 3).

It appeared, therefore, that, depending upon the degree of acidity of the environment, pyrazinamide could be highly active or completely inactive with respect to the H37Rv strain of tubercle bacilli growing *in vitro*.

TABLE 2

ACTIVITY OF PYRAZINAMIDE AGAINST H37Rv TUBERCLE BACILLI ACCORDING TO ENVIRONMENTAL ACIDITY

(*Inoculum* of standard size; *medium*, Tween®-albumin; *activity* measured by macroscopically demonstrated bacillary growth at two weeks)

pH	Drug, γ per Ml.							
	503	250	125	63	32	16	8	0
8.0	1+	1+	2+	3+	4+	4+	4+	4+
7.5	1+	2+	4+	4+	4+			4+
7.0	1+	2+	4+	4+	4+	4+	4+	4+
6.5	3+	4+	4+	4+	4+	4+	4+	4+
6.0	0	3+	4+	4+	4+	4+	4+	4+
5.5	0	0	0	0	0	0	+	4+
5.0								0
4.5								0

TABLE 3

ACTIVITY OF PYRAZINAMIDE AGAINST H37Rv TUBERCLE BACILLI ACCORDING TO ENVIRONMENTAL ACIDITY

(*Inoculum* of standard size; medium, oleic-albumin; *activity* measured by macroscopically demonstrated bacillary growth at three weeks)

pH	Drug, γ per Ml.							
	450	200	100	50	25	12.5	6.25	0
8.0	0	0	0	1+	4+	4+	4+	4+
7.5	1+	2+	3+	4+	4+	4+	4+	4+
7.0	2+	2+	4+	4+	4+	4+	4+	4+
6.5	2+	2+	4+	4+	4+	4+	4+	4+
6.0	0	1+	1+	1+	4+	4+	4+	4+
5.5	0	0	0	0	0	0	0	4+
5.0	0	0	0	0	0	0	0	1+
4.5								0

TABLE 4

ACTIVITY OF NICOTINAMIDE AGAINST H37Rv TUBERCLE BACILLI ACCORDING TO ENVIRONMENTAL ACIDITY

(*Inoculum* of standard size; *medium*, oleic-albumin; *activity* measured by macroscopically visible bacillary growth at three weeks)

pH	Drug, γ per Ml.							
	450	200	100	50	25	12.5	6.25	0
8.0	2+	2+	3+	4+	4+	4+	4+	4+
7.5	4+	4+	4+	4+	4+	4+	4+	4+
7.0	4+	4+	4+	4+	4+	4+	4+	4+
6.5	4+	4+	4+	4+	4+	4+	4+	4+
6.0	0	1+	4+	4+	4+	4+	4+	4+
5.5	0	0	0	0	2+	4+	4+	4+
5.0	0	0	0	0	0	0	0	1+
4.5								0

The effect of changes in environmental acidity on the antimicrobial activity of nicotinamide was studied in the same fashion (table 4). The phenomenon observed with pyrazinamide was also clearly demonstrable with nicotinamide, although higher concentrations of nicotinamide were necessary for complete inhibition of the H37Rv strain at pH 5.5.

The possibility of a relationship between the initial size of the microbial population and these phenomena of acidic environment and pyrazinamide susceptibility was investigated with the H37Rv strain of tubercle bacilli. Ten times the number of tubercle bacilli customarily used for drug-susceptibility tests in this laboratory were used. In contrast to the results with the standard inoculum, when the large populations were introduced in the absence of pyrazinamide or nicotinamide, an abundant growth of tubercle bacilli developed at all levels of acidity tested, including pH 4.5. With this larger initial population, however, the high degree of pyrazinamide susceptibility demonstrable with the standard-sized inoculum was not detectable at all, nor was any nicotinamide susceptibility present.

There is no certain explanation immediately at hand of how the tenfold increase in the initial microbial population resulted in such marked alteration in both growth capacity and drug susceptibility. There is some indication from studies now in progress that both phenomena merely reflect a change in the pH of the medium toward neutrality which eventually occurs with microbial growth. Neither phenomena is unprecedented, however, and, as previously observed by others[13] and noted in this laboratory, the size of the microbial inoculum used must be carefully controlled to demonstrate PAS activity *in vitro*.

Attempts to study the influence of environmental acidity on the pyrazinamide and nicotinamide susceptibilities of a strain of bovine origin (Ravenel) have thus far been unsuccessful. For, when the standard-sized inoculum was used, the Ravenel strain did not always show visible growth at pH 6.0 or below and, hence, its drug susceptibility in the highly acidic environments could not be tested. With the tenfold larger inoculum, the Ravenel strain behaved in a fashion identical with that of H37Rv studied in the same conditions. This question of the behavior of strains of bovine origin in the acidic and

[13] BRITISH MEDICAL RESEARCH COUNCIL: Isoniazid trial: Report No. 4, Lancet, 1953, *2*, 217.

TABLE 5

ACTIVITY OF STREPTOMYCIN AGAINST H37Rv TUBERCLE BACILLI ACCORDING TO ENVIRONMENTAL ACIDITY

(*Inoculum*, standard size; *medium*, Tween®-albumin; *activity* measured by macroscopically visible bacillary growth at two weeks)

pH	Drug, γ per Ml.								
	10	5	2.5	1.25	0.63	0.32	0.16	0.08	0
8.0	0	0	0	0	0	0	1+	4+	4+
7.5	0	0	0	0	0	0	4+	4+	4+
7.0	0	0	0	1+	3+	4+	4+	4+	4+
6.5	0	0	1+	4+	4+	4+	4+	4+	4+
6.0	0	1+	3+	4+	4+				4+
5.5	0	1+	2+	4+	4+	4+	4+	4+	4+
5.0									0
4.5									0

pyrazinamide-containing environments is being studied in greater detail. The subject is of particular interest because Dr. Harold White had previously informed the present writers that pyrazinamide had no demonstrable influence on a strain of bovine origin in experimentally infected mice, whereas the drug had definite effectiveness against human tubercle bacilli when used in the same experimental system[14]. Moreover, in the original studies of pyrazinamide in tuberculous guinea pigs by Dessau and associates[6], it is stated that a strain of bovine origin "showed a rather poor response" in comparison with the results with strains of human origin.

Observations were also made of the influence of environmental acidity on the antituberculous activities of isoniazid and streptomycin. The observations represented merely a repetition of various individual experiments performed at one time or another by many investigators including the present writers. As may be seen in table 5, the well-recognized phenomenon of a substantial reduction of the antimicrobial activity of streptomycin at pH 6.0 and 5.5 was again demonstrated. The fact that isoniazid, in similar circumstances, shows neither a decrease nor an increase in antituberculous activity was likewise demonstrated once again.

In the experiments with the standard-sized inoculum, it was observed that the maximal antimicrobial activity of both pyrazinamide and nicotinamide on the H37Rv strain of tubercle bacilli *in vitro* occurred at pH 5.0 to 5.5, whereas little, if any, activity was demonstrable within the physiologic ranges of pH. It is reasonable to infer that this represents the emergence of intrinsically powerful drug activity when the drug is tested in a suitable environment. An alternative possibility exists, namely, that the results represent relatively weak or even nonspecific drug action on tubercle bacilli existing in conditions relatively unfavorable for growth. This does not seem very likely, however, for in the same experimental conditions the tubercle bacilli were able to grow well even in the presence of streptomycin. Moreover, there was also no increase in the susceptibility of the bacilli to isoniazid in these same circumstances.

The pyrazinamide and nicotinamide activation in the acidic environment is a distinctly unusual phenomenon among the antibacterial drugs which have been in general use. The sulfonamides and streptomycin exert their peak activity at or above neutrality and

[14] WHITE, H.: Personal communication.

become progressively less active as the environmental pH is lowered. A similar pattern is displayed by oxytetracycline against *E. coli*, with staphylococci, the drug shows neither a loss nor a gain of activity as the pH is lowered.[15] Information on this point with respect to the other tetracyclines is lacking at present. Isoniazid shows no loss of activity in an acidic environment but neither does it show a gain in such circumstances. Chloramphenicol generally shows a lesser activity below pH 7.0 than above, although the differences are not marked.[15] With penicillin, the situation is unclear for results in direct conflict have been obtained from tests made under different sets of conditions. None of these drugs, except possibly penicillin, exhibits its maximal activity when the environment becomes more acid, and all exhibit some activity, although not necessarily maximal activity in the pH range of 7.0 to 7.5.

Thus, the property of pyrazinamide and nicotinamide of exerting maximal activity within the acid ranges of pH is distinctly unusual. What is even more unusual, however, is the virtually complete absence of activity within the physiologic ranges of pH. This discrepancy between the conditions which obtain under "physiologic" conditions *in vitro* and the inflammatory lesion of an infection has been emphasized by several writers during recent years and particularly by Dubos[16-21]. In a recent paper he stated:

> The protean character of the infectious process thus causes bacilli and drugs to come into contact in areas differing markedly in physicochemical characteristics often isolated one from the other, and in which the prevailing conditions antagonize to a different degree the antimicrobial activity of the various drugs. As drugs are selected on the basis of their ability to function in the normal physiological environment, they are often ineffective within the lesions where conditions are profoundly different from the physiological. It is this fact which is responsible for the difficulty in achieving complete eradication of the bacilli in pulmonary tuberculosis.[19]

The deficiencies of *in vitro* techniques lie not so much in their "artificiality" as in the rigidity with which they are used. This in turn is a consequence of our considerable ignorance concerning the precise conditions operative *in vivo* which it is desired to mimic *in vitro*. In the particular case of pyrazinamide, it has been possible to demonstrate a high degree of activity on tubercle bacilli of human origin in the test tube, in laboratory animals, and in man. The circumstances in which the *in vitro* activity of pyrazinamide or nicotinamide were demonstrated, although "unphysiologic," presumably obtain to some degree *in vivo* in inflammatory and necrotic areas which apparently are usually at pH 6.0 or below. Indeed, within the monocytes which represent one of the principal sanctuaries of tubercle bacilli, isolated areas of acidity as low as pH 3.0 have been demonstrated.[22]

It is not unreasonable, therefore, to assume that the *in vitro* environment in which pyrazinamide is so highly active represents a sketchy replica of certain environments

[15] Eagle, H., Levy, N., and Fleischman, R.: Antibiotics and Chemotherapy, 1952, *2*, 563.

[16] Dubos, R. J.: Bull. New York Acad. Med., 1945, *21*, 27.

[17] Dubos, R. J.: Am. Rev. Tuberc., 1952, *65*, 637.

[18] McDermott, W.: Principles of chemotherapy, part II. Host factors in chemotherapy, in Dubos, R. J.: *Bacterial and Mycotic Infections of Man*, ed. 2, J. B. Lippincott Company, Philadelphia, 1952, p. 744.

[19] Dubos, R. J.: Am. Rev. Tuberc., 1954, *70*, 391.

[20] Wood, W. B., Jr., McLeod, C., and Irons, E. N.: J. Exper. Med., 1946, *84*, 396.

[21] Wood, W. B., Jr.: in *Year Book of Pathology and Clinical Pathology*, The Year Book Publishers, Chicago, Illinois, 1950, p. 17.

[22] Rous, P.: J. Exper. Med., 1925, *41*, 379, 399, 451.

in which it might be present (and acting) within the host. This is not to say that the whole question of pyrazinamide activity *in vivo* is merely a matter of environmental acidity. On the contrary, from what little is known about the biochemical environments of infectious processes, it is clear that they are quite complicated and probably subject to rapid changes in character. Consequently, although the biochemical environment of the lesion is probably responsible for most chemotherapeutic failures, it is unlikely that any one environmental factor, such as the degree of acidity, will represent the sole determinant of the antimicrobial activity of a drug.

It is not yet known whether the experimental system employed in the present studies can be used to investigate the "escape" of tuberculous infections from the influence of pyrazinamide. This question as well as other aspects of the influence of the biochemical environment on the pyrazinamide and isoniazid activity are under study at the present time.

In summary: Tubercle bacilli of the H37Rv strain are highly susceptible to pyrazinamide *in vitro* in a standard drug-susceptibility test modified only by reducing the pH to 5.0 or 5.5. Nicotinamide is likewise active, although to a lesser degree, when tested in this way. When tested within the "physiologic" ranges of pH, tubercle bacilli of the H37Rv strain are resistant to both pyrazinamide and nicotinamide.

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WALSH MCDERMOTT
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(with the technical assistance of KURT STERN)

June 23, 1954

Correspondence

To the Editor of The American Review of Tuberculosis:

May I comment briefly on two subjects under discussion in your May, 1954, issue.

PNEUMOTHORAX INDUCTION

The view expressed by Weiss, namely, that induction usually occurs by accidental lung puncture, seems to be gaining ground.

Owing to the risk of air embolus, which may even occur before air is injected by aspiration of alveolar air into a torn vessel, this method of induction is to be avoided, if possible, and I believe that by using a certain technique most inductions can be accomplished in what I may term the proper manner—although this is impossible to prove. Using a blunt-ended Kuss cannula, I perforate the anesthetized skin and most of the intercostal tissues with a sharp trocar. I then change to a blunt-ended trocar and proceed very slowly through the remaining intercostal tissues until the parietal pleura is perforated, which can nearly always be appreciated by the sensation of puncture; on withdrawing the trocar, air can often be heard to hiss down the cannula owing to the negative intrapleural pressure—the tap is then turned off and negative intrapleural pressures read and the induction proceeded with. Admittedly, this does not prove that air has not also entered from a lung puncture, but this sharp hiss seems to me against this view. Another point which I find difficult to understand is that, if the lung is usually perforated, why should the hole obligingly seal itself when air is passing through it into the pleural space before collapse of the lung is complete? May I suggest, therefore, that it is possible in most cases by using a careful technique to induce A.P. without perforation of the lung and that this should be our aim, and in this I find myself in entire agreement with the views expressed by Vysniauskas in your January, 1954, editorial.

IRREGULAR DISCHARGES FROM SANATORIUMS

Our "psychiatrist-pthisiologist," George Day, has written entertainingly on this subject and Wilmer has replied with comprehensive information relative to irregular discharges from 22 sanatoriums including Colindale, London, from which I published figures in *Tubercle* in June, 1952. My figures relate to 1938–1951 and the correct over-all figure as given by Wilmer for this period is 16 per cent, but it is of interest that the rate had fallen to 5 per cent in the last two or three years. I attributed this improvement mainly to (*a*) the severe pressure on bed accommodation so that admission to the sanatorium became a prize not lightly to be thrown away, and (*b*) the much more effective treatment (due to streptomycin and PAS and surgery), whose results were plainly apparent to the patients.

I regard 5 per cent as about the irreducible number of self-discharges and these are in the main due to the abnormal personalities of the patients concerned, which render them incapable of adaption to communal life, or to domestic crises in the patient's home which he considers demand his presence.

I take the view that, in this country, high self-discharge rates are an indication of the inefficiency of the sanatorium.

So far this year, there is a tendency for the irregular discharge rate here to rise somewhat, and one factor concerned may be the diminishing pressure on accommodation

COLINDALE HOSPITAL
LONDON, ENGLAND

Sincerely yours,

W. E. SNELL

June 27, 1954

American Trudeau Society

Medical Section of the National Tuberculosis Association

A Statement by the Committee on Therapy Concerning the Treatment of Tuberculous Meningitis

Tuberculous meningitis, once universally fatal, has yielded progressively to improved methods of treatment which have been introduced since streptomycin was first put to clinical use in 1945. The present high level of survival has been reached in a series of steps corresponding roughly with the appearance of three important chemotherapeutic agents. Thus, in 1947, with streptomycin alone, survival rates ranging from 18 per cent to 25 per cent were seen; these rose to levels of 45 per cent to 60 per cent after 1949, when PAS was combined with streptomycin; and, since 1952, rates of 75 to more than 90 per cent survival have been reported coincident with the introduction of isoniazid.

In spite of these impressive statistics, tuberculous meningitis remains a serious disease which must be diagnosed early and treated promptly and adequately if good therapeutic results are to be obtained. Until the disease is brought under control, the patient's status is always critical and the prognosis guarded. Current therapeutic successes tend to obscure completely or at least minimize the importance of a number of background factors which have a variable effect on prognosis. Three of these factors are probably most significant: (*1*) the stage of meningitis that has developed when diagnosis is made and treatment is started, (*2*)[1] the presence of important active tuberculosis elsewhere in the body, and (*3*) the age of the patient.

The stage of meningitis is probably the single most important factor. Untreated tuberculous meningitis may be observed to pass through three phases, and each phase may last for several weeks. Typically, the onset is insidious with mild symptoms of moderate headache, malaise, inattention, lassitude, and moderate afternoon fever. The second phase represents an increase of these symptoms, with the appearance of stiff neck and marked irritability. Nausea and vomiting assume an important role. In children, the vomiting may be projectile. Photophobia is noted, and transient shifting paralysis of the extremities is characteristic. The third phase is characterized by diminishing irritability and increasing coma. It becomes increasingly difficult to arouse the patient as the disease progresses to fatal termination. Considerably less favorable results are observed when third-phase meningitis cases are compared with less advanced cases treated with similar measures.

The presence of important associated active tuberculosis elsewhere in the body, whether pulmonary or extrapulmonary, i.e., renal, bone and joint, or miliary, tends to influence unfavorably the outcome of treatment.

The age of patients was formerly more important than now. This was especially true in adults in middle and old age, among whom failures were common before the advent of isoniazid. Currently, the incidence of meningitis and mortality are highest in the age group from one to four years.

Early diagnosis of tuberculous meningitis is of primary importance if the results of therapy are to be improved. A lumbar puncture should be done whenever there is a suspicion of meningitis. The changes observed in the fluid are those of a serous meningitis with cell

[1] Miliary tuberculosis may progress to involve the meninges despite treatment with streptomycin or streptomycin-PAS. This does not occur when isoniazid is administered. Routine treatment of all miliary cases with isoniazid therefore seems imperative.

counts varying from 20 to several thousand per cu. mm., a lowered sugar and chloride determination, and elevation of protein. These findings, together with the clinical picture, should be sufficient to start treatment. Every effort should be made to demonstrate the presence of tubercle bacilli in the fluid, but treatment should not be delayed until this is accomplished.

Treatment depends principally on chemotherapy. Present knowledge indicates that isoniazid should *always* be tried regardless of what other agent is used. Because of past experience and in view of the critical nature of the problem, it is common practice to use streptomycin, PAS, and isoniazid all together. While successful therapy with isoniazid either alone or in combination with only one of these agents has been reported, it is suggested that all three be used in most circumstances. The greater effectiveness of isoniazid makes practical a reduction of the previously high doses of streptomycin, thus reducing the hazards of eighth nerve damage, and, furthermore, makes intrathecal injection of streptomycin unnecessary except under the most unusual circumstances. Injectable forms of isoniazid and PAS may be used in severely ill or comatose patients until they have sufficiently recovered to take medication by mouth.

The following dosages are recommended in the treatment of meningitis: isoniazid, 8 to 10 mg. per kg. per day for the first week to ten days, then in a dosage of 4 to 6 mg. per kg. per day thereafter; streptomycin, 1 gm. per day for at least three or four months, followed by a reduction to 1 gm. every third day or twice weekly in both children and adults. In small infants, it may be wise to reduce the streptomycin dose to 0.5 gm. per day, but it must be stressed that children tolerate streptomycin better than do adults; PAS is given in doses of 12 gm. per day for adults, 8 to 10 gm. for children, and 4 to 6 gm. for infants. The total duration of treatment is empiric. A minimum of eighteen months to two years of treatment with combined therapy would seem acceptable in the current state of our knowledge.

The *clinical course* of the meningitis is variable. If response is favorable, not only will the symptoms abate, but the cerebrospinal fluid findings will begin to revert toward normal within a few weeks. If recovery is accomplished, cerebrospinal fluid findings should become normal. Routine monthly lumbar puncture for observation of these changes is recommended.

Continued elevation of protein with poor clinical response may indicate the presence of basal meningitis. Basal block with ultimate hydrocephalus and death may develop. Caution is advised against precipitate action in the use of extra measures to relieve block before at least a week's trial of chemotherapy.

To relieve *basal block*, several additional procedures have been used. The most heroic is the use of PPD intrathecally in minute doses. The amount of PPD injected every third day is doubled until a reaction occurs, when it is reduced and then repeated in ascending dosage until reaction again occurs. This process is continued until the block is relieved or the treatment fails. Skull trephine is a necessary preamble, and close follow-up with prompt decompression through ventricular tap may be necessary after any one of the injections of PPD. This method requires adequate neurosurgical facilities and is too complicated and dangerous for routine use.

The use of enzymes such as streptokinase and streptodornase intrathecally to relieve block has been apparently successful in some instances, but like PPD therapy requires careful attention by persons familiar with enzymatic therapy.

Cisternal tap is a safe and useful maneuver in relief of block in children, although it is not valuable in adults. It can be used early whenever suspicion of block exists and may be alternated with lumbar punctures yielding good results in moderate to marked degrees of block.

Recent limited observations indicate that corticotropin and cortisone may have a useful role in some cases of block. These substances should only be used when one is thoroughly convinced of its necessity, and the dosage should be adequate and the duration of treatment as brief as possible.

Other Considerations

The meningitis patient is a serious nursing problem until coma is overcome and cooperation is possible. In children, vomiting is a major problem even under modern chemotherapy. Approximately 50 per cent will require nasal feedings at some time in the early months of treatment, but continuous drip is rarely needed.

Spasticity of the back and extremities is less frequent when intrathecal medication is avoided. Relief of these symptoms is usually obtained by lumbar puncture. Occasionally, when isoniazid is used for several months, especially in children, the patient may show mental confusion, catatonic states, and display unprovoked weeping. Under the circumstances isoniazid is thought to be responsible, and withdrawal for a matter of a few weeks results in recovery from these symptoms.

Prognosis

With isoniazid the prognosis is greatly improved, but some patients still die principally because of advanced disease when first seen. Most deaths occur within the first two weeks of treatment. If patients survive twenty-one days of therapy, they may live indefinitely.

Summary

1. Tuberculous meningitis is still a serious disease, but with improvements in therapy now 90 per cent or more of patients are surviving.

2. The most important single drug in treatment is isoniazid.

3. Duration of treatment is empiric, but a minimum of eighteen months to two years is suggested.

4. Early diagnosis of meningitis remains paramount, although associated other tuberculosis and age may unfavorably modify the results.

5. Basal block which will not spontaneously be relieved by present-day treatment is difficult to anticipate. Relief of block by cisternal tap alternating with lumbar puncture is suggested for children. Occasionally corticotropin or cortisone may be tried in this condition. Other methods such as PPD injection or enzyme installation are thought to be too dangerous or ineffective for general use.

6. Patients alive after twenty-one days of therapy have an excellent chance of surviving indefinitely.

Prepared for the Committee on Therapy
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NOTES

MANUAL FOR CONSECUTIVE CASE CONFERENCE

(Pembine-Type)

The National Tuberculosis Association and the American Trudeau Society have received numerous requests for assistance in planning and conducting conferences similar to the Pembine Conference. The Committee on Therapy of the American Trudeau Society has prepared this *Manual for Consecutive Case Conferences* to assist any groups who may be considering such a conference and for general information as to what such a conference is.

The material in the *Manual* is presented in considerable detail for those who are organizing their first consecutive case conference. The tabular form will provide a ready reference for those who may be more familiar with this type of conference. Each section is concerned with some one phase of planning and organizing.

Part I is devoted to the Pembine Conference, its historical background and methods. Examples of case presentation forms and agenda are included.

Part II states the objective of a consecutive case conference. Basically, these are the presentation of consecutive cases and a critical evaluation of the procedures used.

Part III is concerned with the planning of a conference. Subjects presented include the general organization of the steering committee, methods of financing, selection of those attending and participating, planning and developing the program, and the selection of the cases to be presented.

Part IV outlines the facilities which may contribute to a more successful conference. Suggestions for the displaying of X-rays and lantern slide material are presented. Also, the methods that can be used for recording the proceedings of a conference are discussed.

Part V describes the manner in which a conference is conducted.

Part VI discusses the means by which the information gained at the conference can be made available to others.

Copies of this manual can be obtained from the American Trudeau Society, 1790 Broadway, New York 19, New York.

ANNUAL MEETING OF THE EASTERN TRUDEAU SOCIETY

The 1954 annual meeting of the Eastern Trudeau Society will be held at the Sheraton Park Hotel, Washington, D. C., on October 22–23, 1954.

ANNUAL MEETING OF THE MISSISSIPPI VALLEY TRUDEAU SOCIETY

The 1954 annual meeting of the Mississippi Valley Trudeau Society will be held at the Town House, Kansas City, Kansas, on October 7–9, 1954.

LABORATORY REFRESHER TRAINING COURSES

The following Laboratory Refresher Training Courses will be offered by the Communicable Disease Center during 1954–1955:

Laboratory Diagnosis of Bacterial Diseases
Enteric Bacteriology—8.50
October 18–29, 1954
Laboratory Methods in Medical Mycology
Part 1. Cutaneous Pathogenic Fungi—8.15
November 1–12, 1954

Part 2. Subcutaneous and Systemic Fungi—8.16[1]
November 15–26, 1954
Laboratory Diagnosis of Tuberculosis—8.55
November 15–26, 1954
Laboratory Methods in the Study of Pulmonary Mycoses—8.17
November 29—December 10, 1954
Laboratory Diagnostic Methods in Veterinary Mycology—9.40
December 13–17, 1954
Laboratory Diagnosis of Rabies—8.26[2]
March 14–18, 1955
Laboratory Diagnosis of Viral and Rickettsial Disease—8.20[2]
March 14–25, 1955
Laboratory Diagnosis of Malaria—8.05[3]
Virus Isolation and Identification Techniques—8.21[3]
Laboratory Diagnosis of Influenza—8.25[3]
Typing of *Corynebacterium diphtheriae*—8.42[3]
Special Problems in Enteric Bacteriology—8.51[3]
Phage Typing of *Salmonella typhosa*—8.52[3]

CONFERENCE ON SILICOSIS AND OCCUPATIONAL CHEST DISEASES

A conference on silicosis and occupational chest diseases jointly sponsored by the McIntyre Research Foundation of Toronto, Canada, and the Saranac Laboratory of Saranac Lake, New York, has been arranged for Monday, Tuesday, and Wednesday, February 7, 8, and 9, 1955, in the Town Hall at Saranac Lake.

Both of these organizations have for many years been conducting research along parallel lines. It is a significant development in the field of research that they have decided to pool their resources for this conference. The papers to be presented in the five full sessions will all report on original work conducted or sponsored by either the McIntyre Research Foundation or the Saranac Laboratory. In addition, there will be papers presented by guest lecturers.

Doctors, scientists, and businessmen concerned with the problems of occupational chest diseases in all parts of the United States, Canada, and foreign countries are invited to attend.

Dr. Anthony J. Lanza, formerly director of the Institute of Industrial Medicine and now emeritus professor of Industrial Medicine at New York University-Bellevue Medical Center, has been named chairman of the conference. He is also chairman of the scientific program committee, which comprises Dr. Dudley Irwin, Medical Director, Aluminum Company of America, Pittsburgh, Pa.; Dr. Guy Hannon, Medical Director, McIntyre Research Foundation, Washington, Pa.; Dr. Gordon M. Meade, Executive Director of Trudeau-Saranac Institute; and Dr. Gerrit W. H. Schepers, Director of the Saranac Laboratory.

[1] Completion of course 8.15 or equivalent education or experience is a prerequisite.

[2] Courses 8.26, 8.20, 8.21, and 8.25 will be presented at the Virus and Rickettsia Laboratories, Montgomery, Alabama. Other courses listed will be presented at the laboratories at Chamblee, Georgia.

[3] Courses presented by Special Arrangement.

The roster of course 8.20 will close six weeks prior to the beginning of the course.

The business arrangements, including reservations, will be handled by Norman R. Sturgis, Jr., and the treasurer will be Clarence L. Wagner, both of the Trudeau-Saranac Institute staff. All communications concerning the conference should be addressed to Mr. Sturgis, Saranac Laboratory, Saranac Lake, New York.

RESEARCH FELLOWSHIPS OF THE INTERNATIONAL CHILDREN'S CENTRE

A number of fellowships for the academic year 1954–1955 will be available for research workers who may wish to work at the laboratories of the International Children's Centre, Paris.

At present the program of research of the Centre is essentially connected with problems of antituberculosis vaccination and antipertussis immunization.

The grants amount to 60.000 French francs per month.

Traveling expenses from their residence to Paris will have to be borne by the research fellows.

Research workers who wish to apply for a fellowship are requested to send their application together with their curriculum vitae, record of previous work, and testimonials of their chiefs of service to Professor Bugnard, International Children's Centre, Château de Longchamp, Paris 16°.

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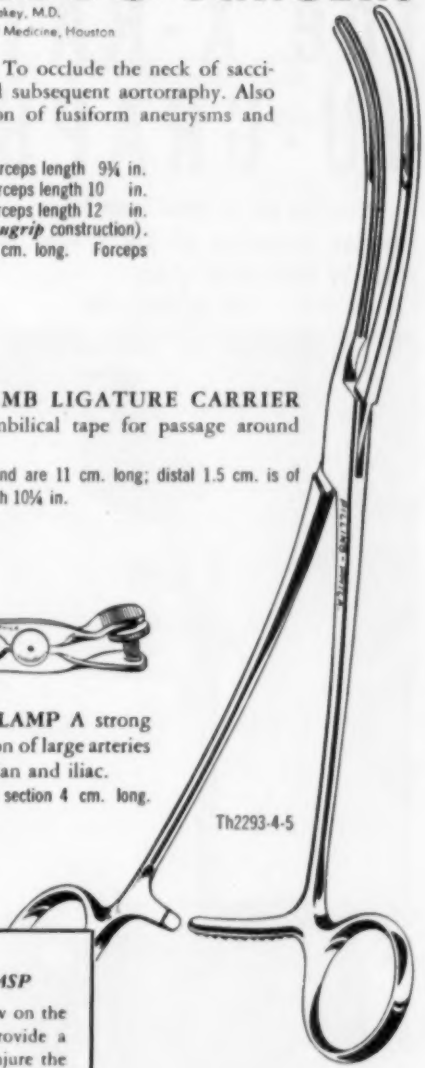

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1. Hobby, G. L., Lenert, T. F., Rivoire, Z. C., Donikian, M., and Pikula, D.: Am. Rev. Tuberc. 67:808, 1953.

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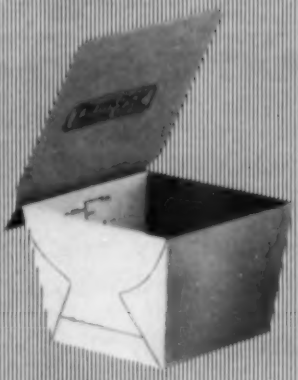

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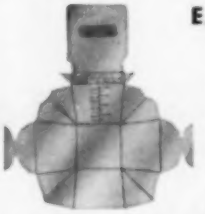

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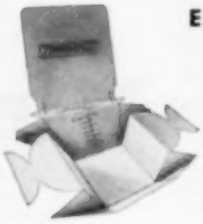

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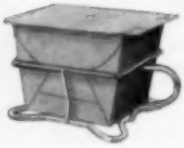

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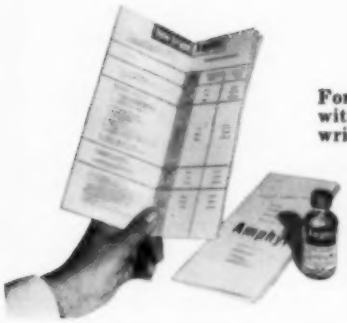
[1] *Smith, C. R.: Disinfectants for tuberculosis hygiene, Soap and Sanitary Chemicals 27:139 (Sept.) 1951; 27:145 (Oct.) 1951.*

[2] *Klarmann, E. G.; Wright, E. S. and Shternov, V. A.: Prolongation of the antibacterial potential of disinfected surfaces, Applied Microbiology 1:19 (Jan.) 1953.*



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